6 - DNA Replication

When James Watson and Francis Crick determined the structure of the DNA double helix, they noticed that the structure provided clues to how DNA is copied prior to cell division. This copying process is called **DNA replication** (see **figure 6.1**).

Overview of DNA Replication

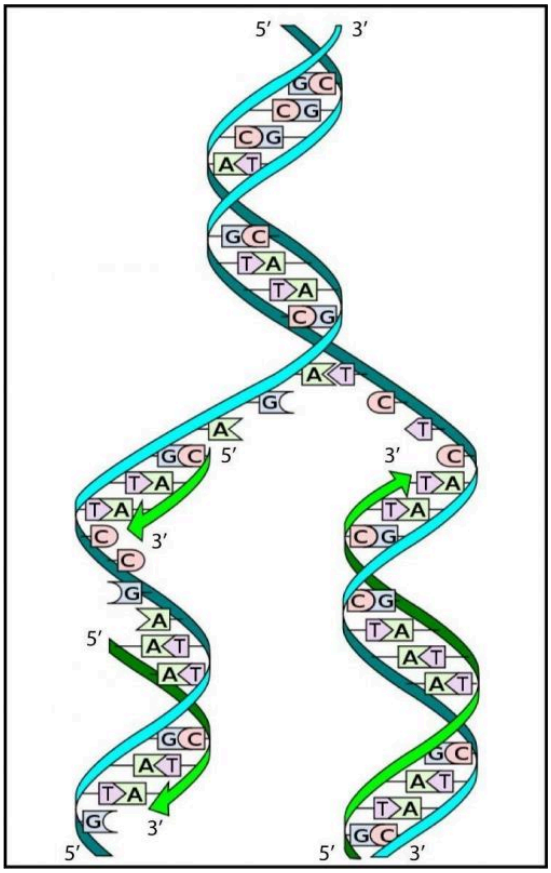

Figure 6.1 ***Overview of DNA Replication*** *--- Image created by SL*

Watson and Crick proposed that during DNA replication, the two original DNA strands within the double helix separate, and two new strands of DNA are synthesized. The two original DNA strands are called **template DNA strands** or **parental DNA strands**; each of the newly synthesized DNA strands is called a **daughter DNA strand**.

When DNA nucleotides (deoxyribonucleoside triphosphates or **dNTPs**) are used to generate the daughter DNA strands, the AT/GC rule is followed. Hydrogen bonds are formed between the nitrogenous bases within the incoming nucleotides and the template strand nitrogenous bases. Then a phosphodiester bond is formed between the free 5' phosphate on the incoming nucleotide and the free 3' hydroxyl group on the growing daughter DNA strand. The dNTPs used as the

substrates for DNA synthesis include **deoxyadenosine triphosphate (dATP)**, **deoxythymidine triphosphate (dTTP)**, **deoxycytidine triphosphate (dCTP)**, and **deoxyguanosine triphosphate (dGTP)**.

Key Questions

- What is a template (parental) DNA strand?
- What is a daughter DNA strand?
- What are the four dNTPs used in DNA replication?

A. DNA Replication in Bacteria

Origin of Replication in Bacteria

The site on the bacterial chromosome where DNA replication begins is the **origin of replication** (see **figure 6.2**). The bacterium *E. coli* has a single origin of replication called ***OriC***. *OriC* is a 275 base pair (bp)-long region that contains important DNA sequences, including:

- **AT-rich sequences**. These AT-rich sequences are significant as only two hydrogen bonds hold AT base pairs together in DNA. Less energy is required to separate AT-rich DNA sequences than GC-rich sequences, so the parental DNA strand separation that is required during DNA replication initiates at these AT-rich sequences.
- **DnaA box sequences**. The DNA replication protein **DnaA** binds to the DnaA box sequences to initiate template DNA strand separation. Template DNA strand separation occurs at the AT-rich sequences.
- **GATC methylation sequences**. Methylation of the adenine bases within each GATC methylation sequence serves as an activation signal for DNA replication.

DNA replication begins at *OriC* and proceeds in both directions (clockwise and counterclockwise) around the circular bacterial chromosome (**bidirectional replication**). Further, a **replicon** is defined as all of the DNA replicated from a single origin. Since the entire *E. coli* chromosome is replicated from a single origin, the chromosome is one replicon**.**

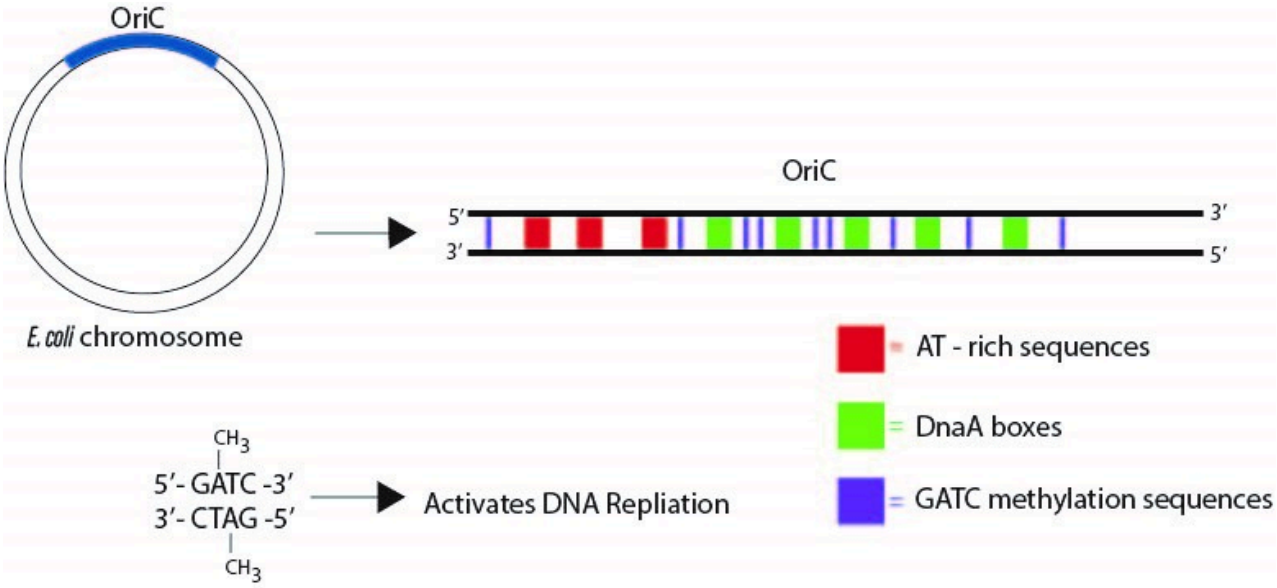

Figure 6.2 ***OriC in E.coli ---*** *Image created by KMD*

Key Questions

- What are the names and functions of the three DNA sequence types found in *OriC*?
- What is a replicon?

Replication Initiation

The steps involved in DNA replication in bacteria are (see **figure 6.3**):

1. **DnaA proteins bind to the DnaA box sequences**. When DnaA proteins bind to ATP, DnaA binds tightly to the DnaA box sequences within *OriC*.
2. **The origin forms a loop and the individual DNA strands separate.** Multiple copies of the DnaA bind to each other, forming a loop in the DNA. The DNA loop promotes DNA strand separation within the AT-rich sequences of *OriC*. This looping of the DNA and strand separation requires ATP cleavage by the DnaA protein. After ATP is cleaved, the DnaA proteins are released from *OriC*.
3. **A copy of DNA helicase binds to each of the two separated DNA strands.**
4. **The DNA helicases move along the template DNA strands, separating the DNA strands to form two replication forks**. Template DNA strand separation starts at *OriC* and moves in both directions around the circular bacterial chromosome. DNA helicase cleaves ATP and uses the released energy to catalyze DNA strand separation.
5. **Single-stranded DNA binding proteins (SSBPs) bind to the separated single-stranded template DNA strands.** SSBPs prevent the template DNA strands, separated by DNA helicase, from reforming hydrogen bonds, so that DNA replication can proceed.

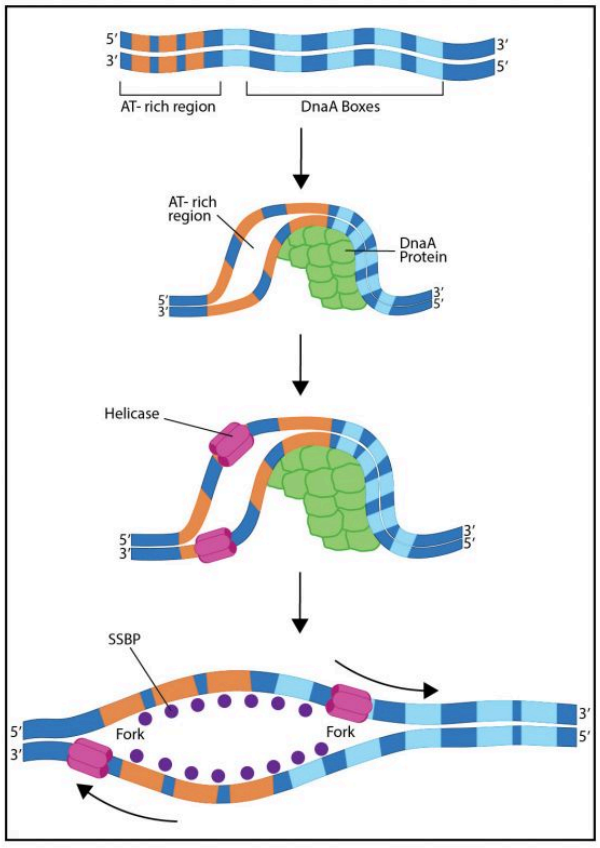

Figure 6.3 ***Replication Initiation in Bacteria*** *--- Image created by SL*

Coordinating DNA Replication with Cell Division

Most bacteria divide quickly; for example, the cell division time of *E. coli* is approximately 20 minutes. If DNA replication in *E. coli* does not keep up with the division of the cytoplasm, daughter cells will be formed that lack chromosomes. On the other hand, if DNA replication occurs too quickly, daughter *E. coli* cells would contain more than one copy of the chromosome.

How is DNA replication and division of the cytoplasm coordinated? *E. coli* coordinates these two processes by regulating how often DNA replication starts. There are two general ways to regulate the initiation of DNA replication:

- **Limiting the amount of active DnaA protein**. To initiate DNA replication, DnaA proteins must be bound to all DnaA box sequences within *OriC*. When a bacterial cell decides to replicate the DNA, there is only enough active DnaA proteins in the cell to bind to the DnaA box sequences within a single copy of *OriC*. After DNA replication occurs, there are now two copies of chromosome (and two copies of *OriC)* in the same cell. At this point, there is not enough active DnaA protein present in the cell to start a second round of DNA replication. By the time additional copies of the DnaA proteins are synthesized, the cytoplasm has divided producing two daughter cells.
- **Methylating GATC sequences**. The enzyme **DNA adenine methyltransferase (Dam)** recognizes the GATC methylation sequences in *OriC* and methylates the adenine nitrogenous bases in both DNA strands. Recall that there are numerous GATC methylation sequences in *OriC*. If every GATC sequence is methylated, DNA replication is initiated. After DNA replication, two DNA molecules are found in the same bacterial cell. Within each of these two molecules, the parental DNA strands contain methylated adenine, but the daughter DNA strands do not. A new round of DNA replication does not start until the Dam protein methylates the adenines within the daughter DNA strands (this can take several minutes). Thus, an *E. coli* cell has enough time to divide its cytoplasm prior to initiating a second round of DNA replication.

Key Questions

- What are the names and functions of the four proteins involved in DNA replication initiation in *E. coli*?

Replication Elongation

The elongation stage of DNA replication in bacteria consists of the following steps (see **figure 6.4**):

1. **RNA primers are synthesized**. After the template DNA strands have separated, small RNA strands (10-12 nucleotides long) are synthesized that form hydrogen bonds with the template DNA strands. These RNA **primers** provide the free 3’-OH groups required by DNA polymerases to initiate daughter DNA strand synthesis.

2. **DNA synthesis occurs by reading the template DNA strands.** Daughter DNA strands are synthesized in the 5’ to 3’ direction by adding dNTPs to free 3'-OH groups. However, because the template DNA strands are antiparallel to the daughter DNA strands, DNA polymerases read the template DNA strands in the 3’ to 5’ direction as the daughter DNA strands are synthesized. Note that as the DNA polymerase reads the template DNA strand 3' to 5' and synthesizes the daughter DNA strand 5' to 3', the DNA polymerase is moving in a single direction.

   Since DNA polymerases only synthesize the daughter DNA strands in the 5’ to 3’ direction, the two daughter DNA strands synthesized at each replication fork are made in opposite directions. One newly synthesized daughter DNA strand is called the **leading strand.** The leading strand is synthesized in the same direction that the replication fork is moving as the template DNA strands are separated. The leading DNA strand requires only one RNA primer and DNA synthesis is **continuous**. The other newly synthesized daughter DNA strand at each replication fork is the **lagging strand.** The lagging strand is synthesized as a series of **Okazaki fragments** (1000–2000 nucleotide-long DNA fragments) in the opposite direction the replication fork is separating the template DNA strands. Each Okazaki fragment is initiated by a single RNA primer; the lagging DNA strand is synthesized in a **discontinuous** (fragmented) manner.

3. **The RNA primers are removed.** Removing the RNA primers results in a gap between each Okazaki fragment.

4. **DNA synthesis fills the gaps left by the removed RNA primers.** DNA synthesis to fill the primer gaps occurs 5' to 3'.

5. **The adjacent Okazaki fragments are linked (ligated) together.** Ligation of the adjacent Okazaki fragments forms a continuous lagging DNA strand.

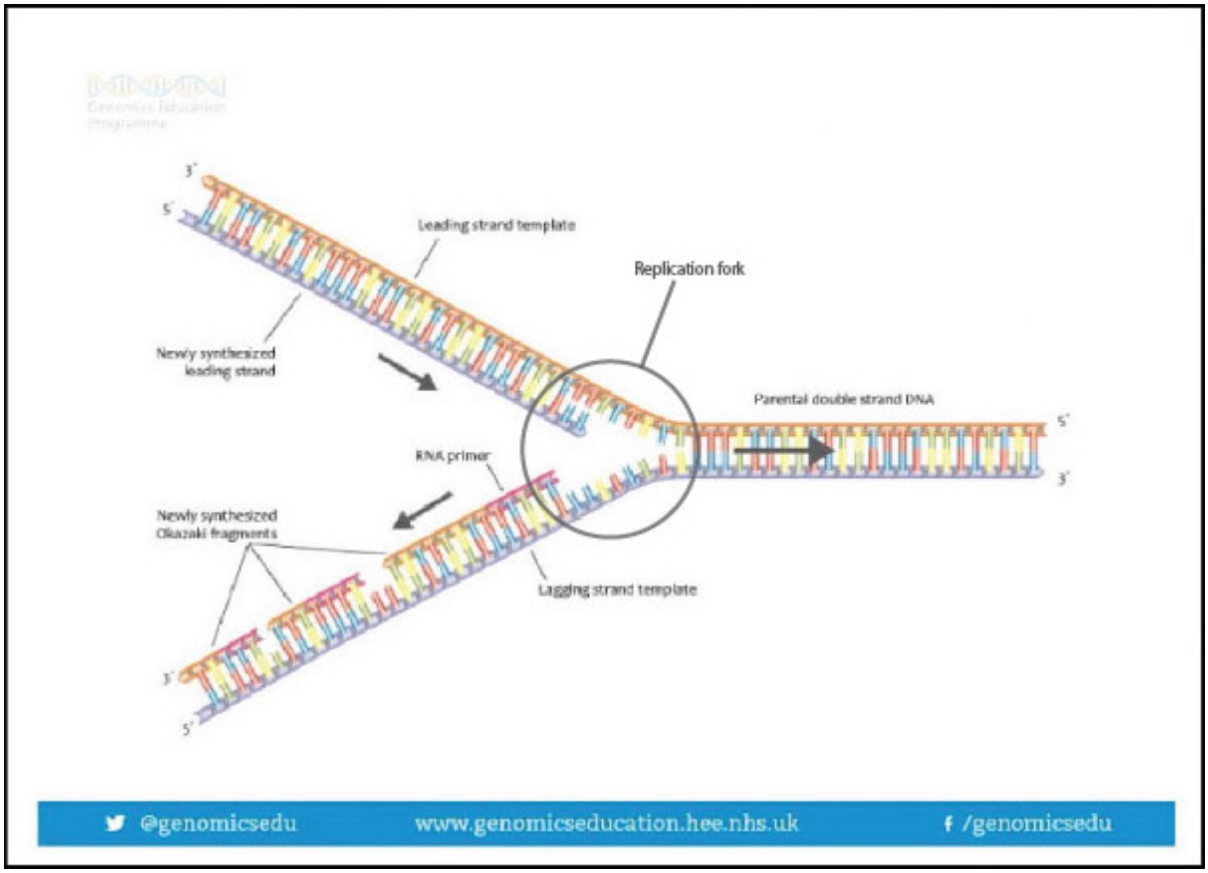

Figure 6.4 ***Replication Elongation ----*** *Image by Genomics Education Programme. Image licesensed under CC BY 2.0*

Key Questions

- What are the major events in the elongation stage of DNA replication in bacteria?
- In what direction do the DNA polymerases synthesize the daughter DNA strands?
- What is the difference between the leading and lagging DNA strands?

Proteins Involved in Elongation

The following proteins are involved in the elongation stage of DNA replication in bacteria (see **figure 6.5**):

- **DNA helicase**. DNA helicase separates the two parental DNA strands as the replication forks proceed from *OriC* clockwise and counterclockwise around the circular *E. coli* chromosome. DNA helicase uses the energy in ATP to break the hydrogens bonds between base pairs as the replication forks proceed.

- **Single-stranded DNA binding proteins (SSBPs).** SSBPs prevent the template DNA strands, separated by DNA helicase, from reforming hydrogen bonds.

- **DNA gyrase.** Since DNA is a right handed double helix, the separation of the parental DNA strands by DNA helicase produces **positive supercoiling** ahead of each replication fork. This positive supercoiling can be lethal to a bacterial cell if left unchecked. DNA gyrase functions to relieve this positive supercoiling by introducing **negative supercoils** ahead of each replication fork. DNA gyrase cleaves ATP and uses the released energy to form negative supercoils.

- **DNA primase.** To synthesize the daughter DNA strands, short RNA **primers** are synthesized by DNA primase. As mentioned earlier, the **leading strand** (DNA synthesis in the same direction as the movement of the replication fork) requires only a single RNA primer, while the **lagging strand** (DNA synthesis in the opposite direction as the movement of the replication fork) requires many RNA primers. Since DNA primase synthesizes an RNA nucleic acid strand (i.e., the primer), DNA primase cleaves RNA nucleotides (e.g., ATP, UTP, CTP, and GTP). As the RNA nucleotides are cleaved by DNA primase, two of the phosphate groups are released, while the remaining nucleoside monophosphates (e.g., AMP, UMP, CMP, and GMP) are incorporated into the synthesized primers.

- **The DNA polymerase III holoenzyme.** The DNA polymerase III holoenzyme synthesizes the daughter DNA strands in the 5′ to 3′ direction. A single DNA polymerase III holoenzyme synthesizes both the leading and lagging DNA strands at each replication fork simultaneously (see below). The DNA polymerase III holoenzyme synthesizes DNA using the nucleotides dATP, dTTP, dCTP, and dGTP as substrates. During daughter strand synthesis, these DNA nucleotides are cleaved, releasing two of the phosphate groups. The remaining nucleoside monophosphates (e.g., dAMP, dTMP, dCMP, and dGMP) are incorporated into the daughter DNA strands.

- **DNA polymerase I.** DNA polymerase I removes the RNA primers and synthesizes DNA to fill in the sequence gaps left by the removed primers. DNA synthesis by DNA polymerase I also occurs in the 5′ to 3′ direction. Like the DNA polymerase III holoenzyme, DNA polymerase I uses the nucleotides dATP, dTTP, dCTP, and dGTP as substrates as it synthesizes DNA. These DNA nucleotides are cleaved, releasing two of the phosphate groups. The remaining nucleoside monophosphates (e.g., dAMP, dTMP, dCMP, and dGMP) are incorporated into the synthesized DNA.

- **DNA ligase.** DNA ligase forms the final covalent bond that links adjacent Okazaki fragments into a continuous daughter DNA strand. DNA ligase uses the energy within ATP to synthesize the final covalent bond in the daughter DNA strand.

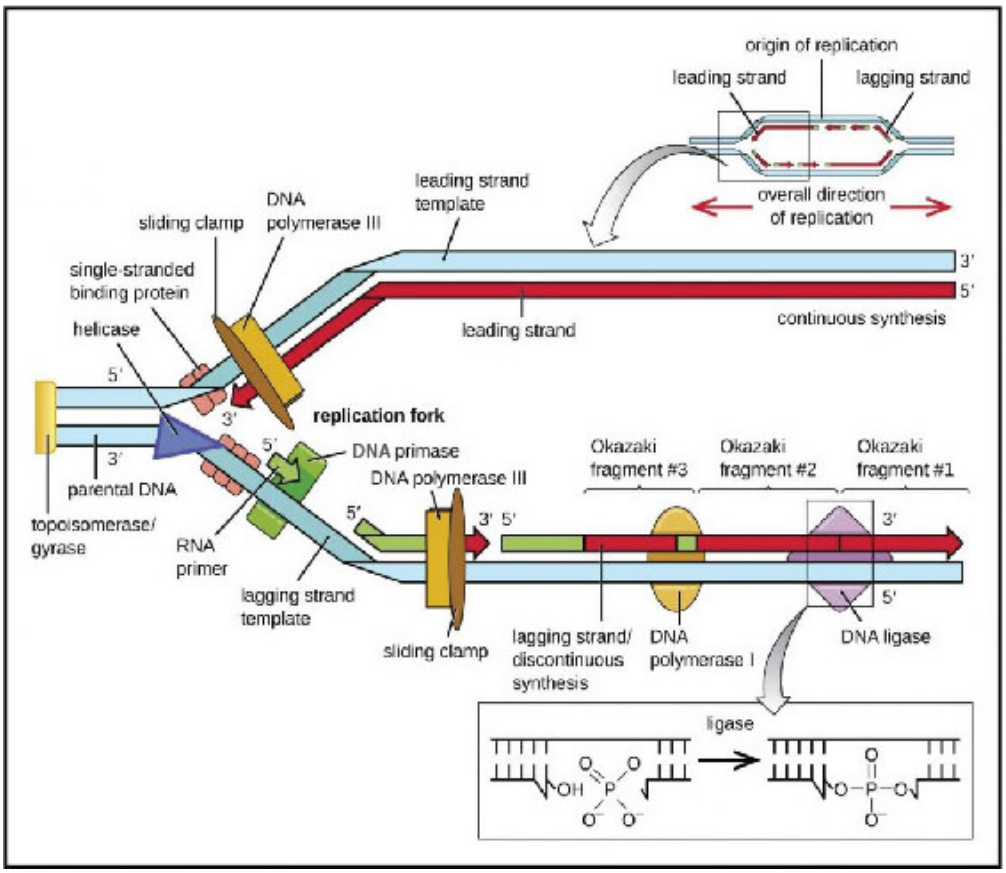

Figure 6.5 ***Bacterial Replication Proteins*** *--- This image is used from OpenStax (access for free at https://openstax.org/books/biology-2e/pages/1-introduction)*

Key Questions

- What are the functions of the seven proteins involved in elongation in *E. coli*?
- List four replication elongation proteins that use ATP as energy.
- List two replication elongation proteins that use dNTPs as substrates for DNA synthesis.

DNA Polymerase III Holoenzyme

DNA polymerase III is a **holoenzyme** (multi-protein enzyme complex) composed of at least ten unique protein types (see **figure 6.6**). Moreover, each of these unique protein types within the DNA polymerase III holoenzyme is present in multiple copies, making the overall composition of the DNA polymerase III holoenzyme quite complex. The protein subunit composition of the DNA polymerase III holoenzyme is as follows:

- **Two alpha (α) protein subunits.** The α protein subunits of the DNA polymerase III holoenzyme carry out the **5' to 3' polymerase** activity to synthesize DNA. One α protein subunit synthesizes the leading DNA strand; the other α protein subunit synthesizes the lagging DNA strand.
- **Four beta (β) protein subunits.** The β protein subunits form sliding clamps that attach the two α subunits to the template DNA strands. These β subunits slide along with the template DNA strands during DNA replication, preventing the α subunits from falling off (increase DNA polymerase III holoenzyme processivity; see below).
- **Two epsilon (ε) protein subunits.** The ε protein subunits of DNA polymerase III possess **proofreading** activity (see below) that fixes mistakes made during DNA replication.
- **Accessory protein subunits.** The accessory protein subunits load the α and β subunits onto the RNA primers during lagging strand synthesis and maintain the overall stability of the DNA polymerase III holoenzyme.

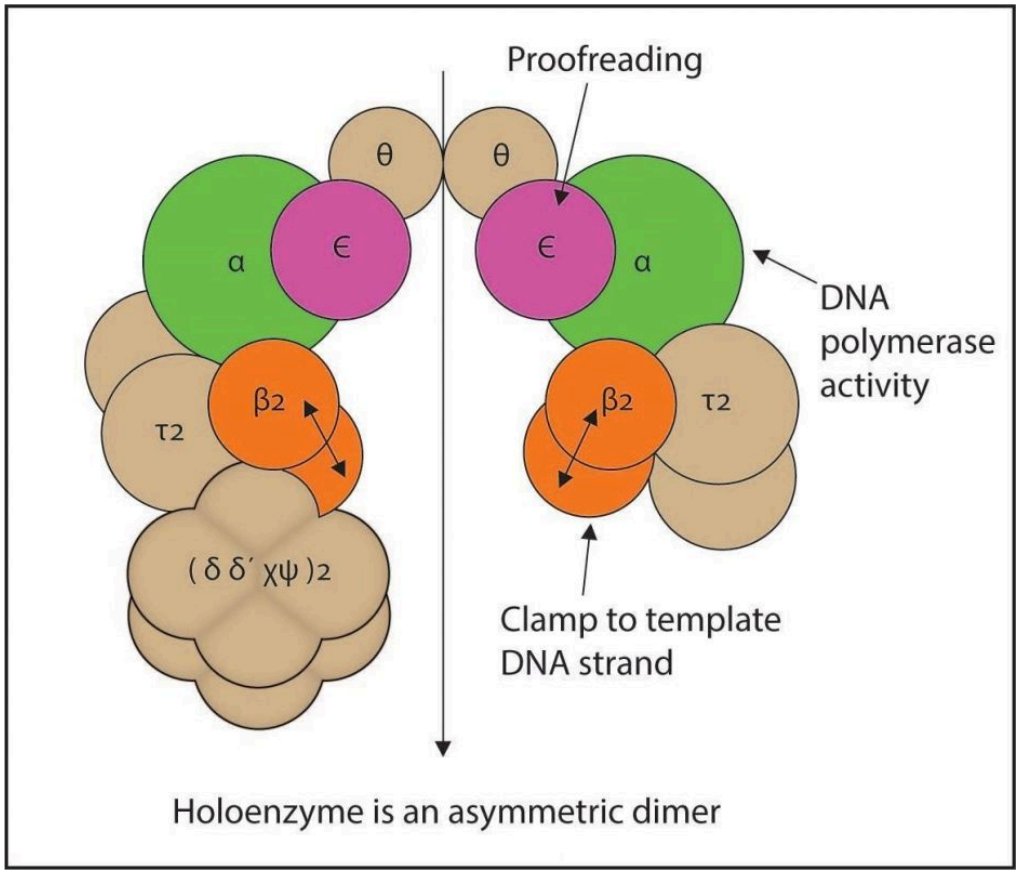

Figure 6.6 ***DNA Polymerase III Holoenzyme.*** *The direction the replication fork is moving is shown by the arrow in the center of the image (i.e. the replication fork is moving from top to bottom) The protein subunits on the left side of the image synthesize the lagging DNA strand; the protein subunits on the right synthesize the leading strand. The α subunits (green), β subunits (orange), ε subunits (pink), and accessory subunits (tan) are indicated. --- Image created by SL*

Key Questions

- What are the functions of the α, β, and ε subunits of the DNA polymerase III holoenzyme?

DNA Replication Proteins form Complexes

Many of the DNA replication enzymes described above are not physically separated. Each enzyme has a distinct function in DNA replication; however, many of these enzymes are physically linked to each other to form multiprotein "machines." For example, the **primosome** is a protein complex formed by DNA helicase and DNA primase. The primosome moves along the DNA separating the DNA strands and simultaneously synthesizing lagging strand RNA primers. Further, the primosome itself is part of a larger multi-subunit complex called the **replisome**. The replisome includes:

- The primosome components (DNA helicase, DNA primase).
- A DNA polymerase III holoenzyme (including the α, β, ε, and accessory protein subunits).

There is a single replisome per replication fork in the bacterium *E. coli*. Since a replicating bacterial chromosome has two replication forks, there are two replisomes per bacterial chromosome.

Key Questions

- What are the protein components of the primosome?
- What are the protein components of the replisome?

DNA Polymerases in Bacteria

In the bacterium *E. coli*, there are five DNA polymerase types. We will focus our attention on DNA polymerases I and III, as these two enzymes are involved in DNA replication. The other three DNA polymerases (DNA polymerase II, IV, and V) are involved in repairing bacterial DNA that has been damaged by environmental agents.

DNA polymerase III (also called the DNA polymerase III holoenzyme; see above) replicates the leading and lagging DNA strands (has **5' to 3' polymerase** activity). DNA polymerase III also contains a **proofreading activity** that removes DNA replication mistakes in the 3' to 5' direction (the so-called **3' to 5' exonuclease** activity; see below). **DNA polymerase I** is composed of a single protein subunit and functions to remove Okazaki fragment RNA primers in the 5' to 3' direction (i.e., the **5' to 3' exonuclease** activity). DNA polymerase I also fills in the gaps left by the removed RNA primers with DNA via its **5' to 3' polymerase** activity and has **3' to 5' exonuclease activity** (proofreading activity; see below).

All DNA polymerases have two unique features. First, DNA polymerases require a free 3'-OH group provided by the primer to begin DNA synthesis. The primer used within cells is RNA; however, DNA polymerases can use DNA primers to synthesize DNA as well. In fact, DNA primers are commonly used when synthesizing DNA in the lab (see Part 8). Second, DNA polymerases synthesize the growing daughter strand in the 5' to 3' direction only.

Key Questions

- What are the two enzymatic activities of DNA polymerase III holoenzyme?
- What are the three enzymatic activities of DNA polymerase I?
- What are two unique features of all DNA polymerases?

DNA Polymerase Mechanism

DNA polymerases use the chemical energy stored within the high energy phosphate bonds of deoxyribonucleoside triphosphate (**dNTP**) molecules to synthesize the daughter DNA strands. Specifically, the DNA polymerase mechanism involves (see **figure 6.7**):

1. The DNA polymerase reads a nitrogenous base in the template DNA strand and binds to the complementary dNTP according to the AT/GC rule. The incoming dNTP forms hydrogen bonds with the nitrogenous base in the template DNA strand.
2. The free 3'-OH group on the growing daughter DNA strand reacts with the phosphate groups on the incoming dNTP.
3. A high energy bond within the dNTP is broken releasing two of the phosphate groups in the form of **pyrophosphate (PP_i)**.
4. The released energy is used to synthesize a new phosphodiester bond between the 3' end of the growing DNA strand and the 5' end of the incoming nucleotide.

The DNA polymerase III holoenzyme is **processive**. Processivity means that the DNA polymerase III holoenzyme can add many nucleotides to a daughter DNA strand without falling off the template DNA strand. This processivity is due to the four β subunits (sliding clamps; see above) found within the DNA polymerase III holoenzyme.

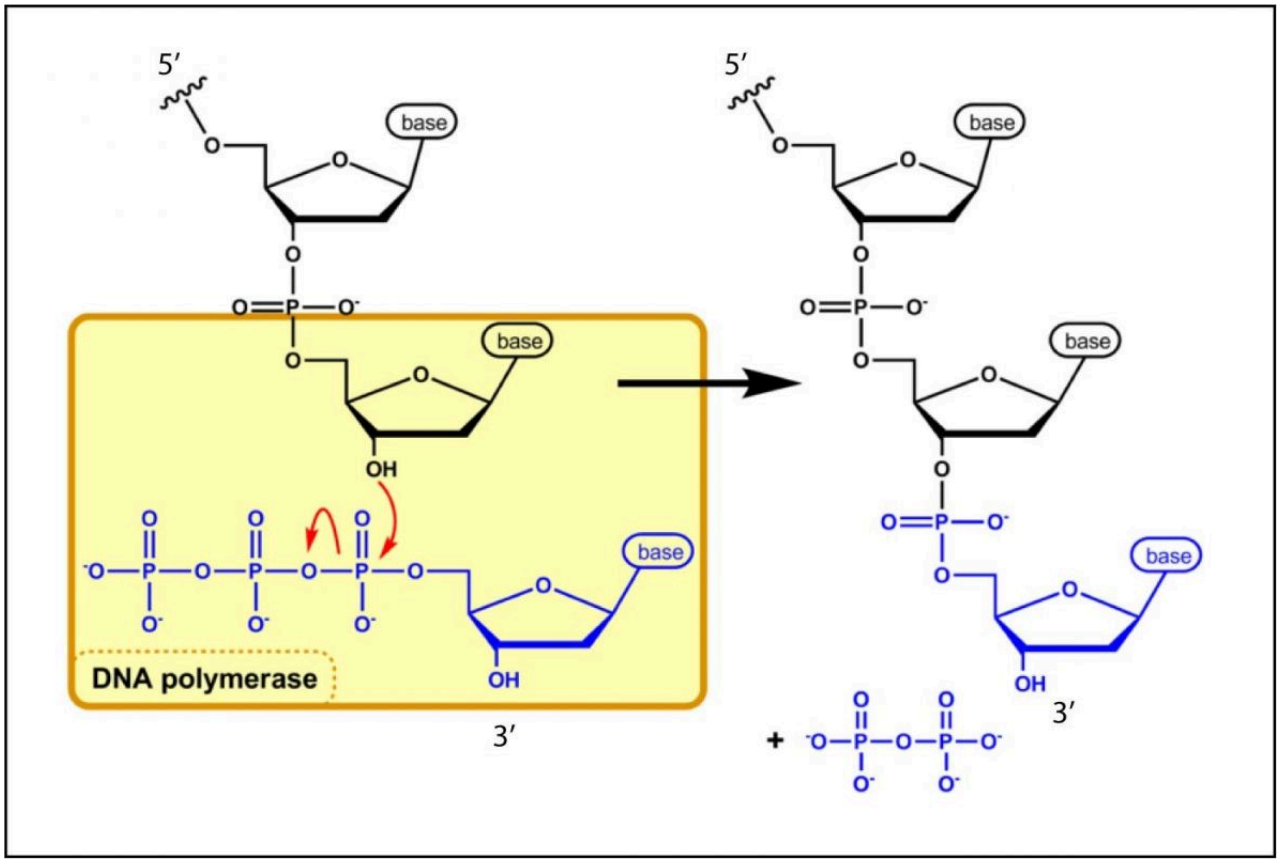

Figure 6.7 ***DNA Polymerase Mechanism*** *--- Image created by Michal Sobkowski and is licensed under CC BY 3.0.*

Key Questions

- Describe the DNA polymerase mechanism.
- What is meant by the phrase "DNA polymerases are processive?"

Proofreading by DNA Polymerases

DNA polymerases incorporate the wrong nucleotide (i.e., a nucleotide that forms base pairs that deviate from the AT/GC rule) into a daughter DNA strand rarely. For example, the DNA polymerase III holoenzyme is thought to incorporate the wrong nitrogenous base once in every 10–100 million nitrogenous bases in a daughter DNA strand. This accuracy during DNA synthesis is called **fidelity**; both DNA polymerase I and the DNA polymerase III holoenzyme are said to have high fidelity (low error rates). The fidelity of DNA polymerases is the combination of three factors:

- **The stability of the hydrogen bonds between AT and GC.** Mismatched nitrogenous base pairs fail to form hydrogen bonds altogether or result in less stable hydrogen bonds.
- **The active site of DNA polymerases is specific.** A covalent bond is not formed between the free 3'-OH group of the growing daughter DNA strand and the free 5' phosphate group of the incoming dNTP unless correct base pairing occurs.
- **Proofreading.** If an incorrect base pair is accidently formed, the DNA polymerase can pause, recognize the mismatch, and remove it (see **figure 6.8**). This **proofreading activity** occurs in the 3' to 5' direction on the daughter DNA strand and is sometimes called the **3' to 5' exonuclease activity** of the enzyme. Once proofreading is complete, the DNA polymerase can resume incorporating dNTPs into the growing daughter DNA strand in the 5' to 3' direction.

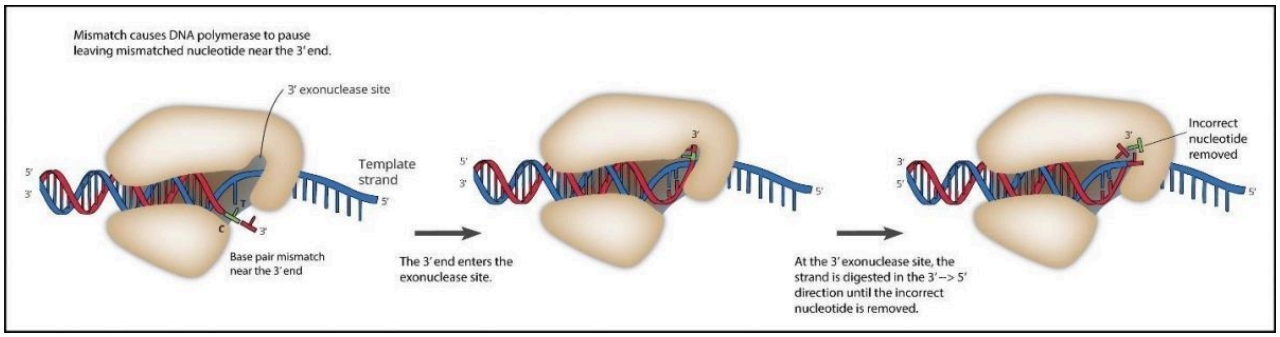

Figure 6.8 ***Proofreading*** *--- Image created by SL*

Key Questions

- What is meant by proofreading?
- Which enzymatic activity is responsible for proofreading?
- What is meant by the phrase, "DNA polymerases display high fidelity?"

Termination of Replication in Bacteria

DNA replication in *E. coli* terminates at specific locations within the circular chromosome called **termination (*ter*) sequences**. Since there are two replication forks moving in opposite directions around the circular chromosome, there are two *ter* DNA sequences. Each *ter* sequence (the **T1** and **T2** sequences) stops the advancement of one of the two replication forks (see **figure 6.9**). Proteins called **termination utilization substances (Tus)** bind to the T1 and T2 sequences. Tus proteins release the replisomes from the two replication forks, terminating DNA replication.

Once replication ceases, **DNA ligase** forms the final covalent bond between the 5' and 3' ends of each daughter DNA strand, resulting in two double-stranded circular *E. coli* chromosomes. These chromosomes can then be distributed to

daughter *E. coli* cells after cell division.

Occasionally, the two chromosomes produced by DNA replication are intertwined like the links in a chain. These intertwined DNA molecules are called **catenanes**. Catenanes must be separated prior to the division of the *E. coli* cytoplasm, so that each daughter cell receives a chromosome. **DNA gyrase** solves this catenane problem by cutting one chromosome (both DNA strands are cut), passing the other chromosome through the break, and sealing the break to generate two separate chromosomes that can be distributed properly to the daughter bacterial cells.

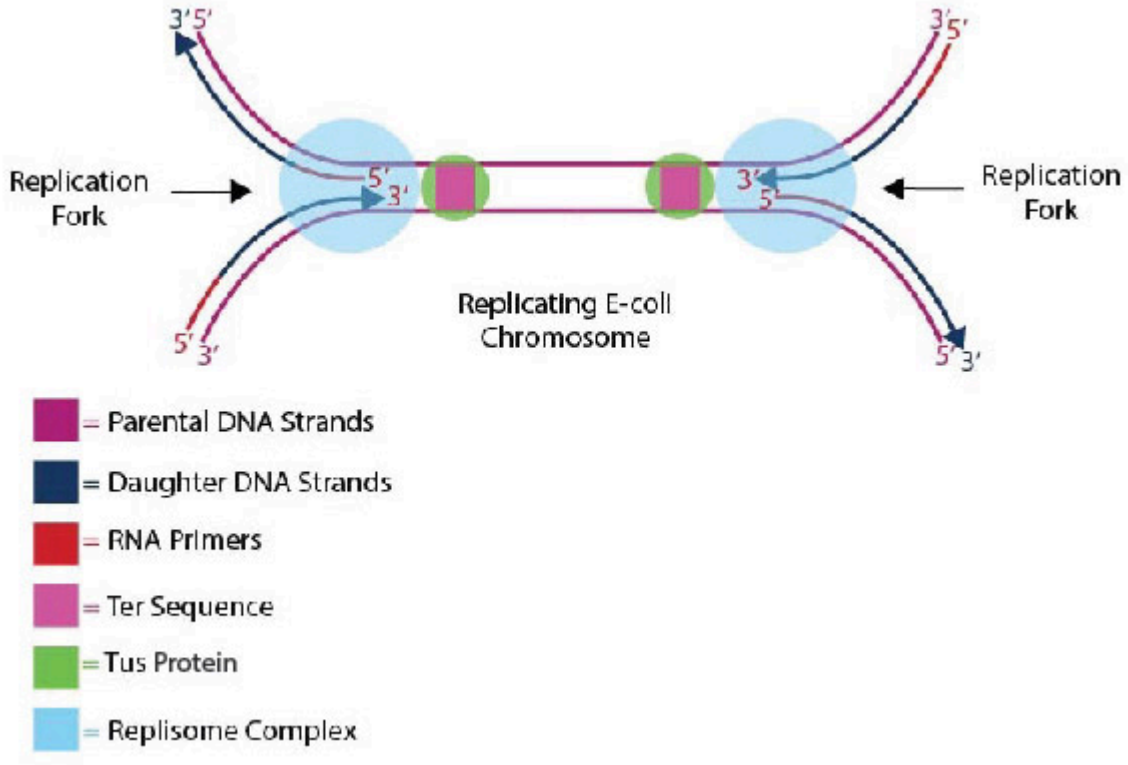

Figure 6.9 ***Termination of Replication in E. coli*** *--- Image created by KMD*

Key Questions

- What DNA sequences participate in replication termination in *E. coli*?
- What are the names and functions of the three proteins that participate in replication termination in *E. coli?*
- How are catenanes resolved?

B. DNA Replication in Eukaryotes

Eukaryotic Origins

Eukaryotic DNA replication is more complex than DNA replication in bacteria. This increase in complexity is because eukaryotic genomes are generally larger than prokaryotic genomes, and the genetic material in eukaryotes is organized into linear chromosomes. However, the good news is that the DNA replication process is similar in prokaryotes and eukaryotes and many of the DNA replication proteins (helicases, primases, and polymerases) identified in bacteria have

eukaryotic counterparts that function in the same way. In contrast, one major difference between prokaryotic and eukaryotic DNA replication is that eukaryotic chromosomes have multiple replication origins (see **figure 6.10**). Like bacteria, DNA replication proceeds bidirectionally from each origin, with the formation of two replication forks per origin. As DNA replication occurs, the replication forks from adjacent origins fuse, eventually producing two identical sister chromatids.

In a model eukaryotic organism, the bread yeast *Saccharomyces cerevisiae*, the 250–400 origins are called **ARS elements.** *S. cerevisiae* ARS elements have the following features:

- ARS elements are approximately 50 base pairs (bp) in length.
- ARS elements are AT-rich. The presence of numerous AT base pairs within the origin promotes DNA strand separation.
- ARS elements contain an **ARS consensus sequence (ACS)**. This ARS consensus sequence is the binding site for the ORC protein complex (see below).

The DNA replicated from a single ARS element is called a **replicon**. Since eukaryotic organisms have many origins, eukaryotes also have many replicons. For example, *S. cerevisiae* contains 250–400 replicons per genome, while the human genome is thought to contain approximately 25,000 replicons.

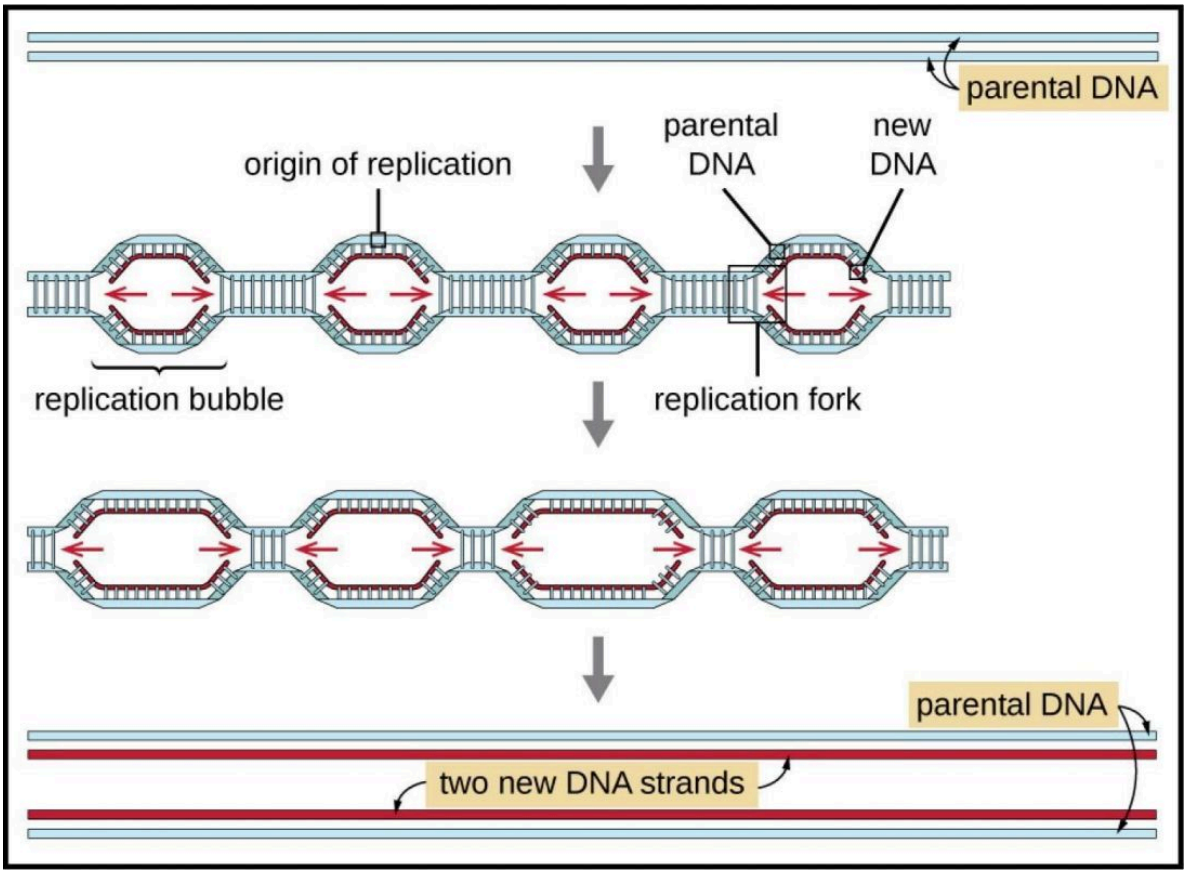

Figure 6.10 ***Eukaryotic Chromosomes Have Multiple Origins*** *--- This image is used from OpenStax (access for free at https://openstax.org/books/biology-2e/pages/1-introduction)*

Key Questions

- How is DNA replication in prokaryotes and eukaryotes similar?
- What is one major differences between prokaryotic and eukaryotic replication?
- What are the features of an ARS element?

Replication Initiation in Eukaryotes

A multi-subunit **prereplication complex (preRC)** assembles on each ARS element and initiates DNA replication in eukaryotes (see **figure 6.11**). The preRC contains the following protein components:

- The **origin recognition complex (ORC)**. ORC binds directly to the ARS consensus sequence within each ARS element (origin).
- **Regulatory proteins**. Two regulatory proteins, **cdc6** and **cdt1**, bind to ORC and function to inhibit the initiation of DNA replication during the G_1, G_2, and M phases of the cell cycle. That way the initiation of DNA replication is tightly controlled; DNA replication can occur only during the synthesis (S) phase of the cell cycle. During S phase, cdc6 and cdt1 are phosphorylated by cellular kinases, causing cdc6, cdt1, and ORC to be released from the ARS element. DNA replication is then initiated.
- **MCM helicase.** Once the cdc6, cdt1, and ORC proteins are released, the MCM helicases catalyze the separation of the two parental DNA strands forming two replication forks. Like prokaryotic DNA helicases, the MCM helicases cleave ATP and use the released energy to form replication forks.

After the DNA strands have separated, **replication protein A (RPA)** prevents the separated DNA strands from reforming hydrogen bonds. The eukaryotic DNA polymerases can then begin the elongation stage of DNA replication.

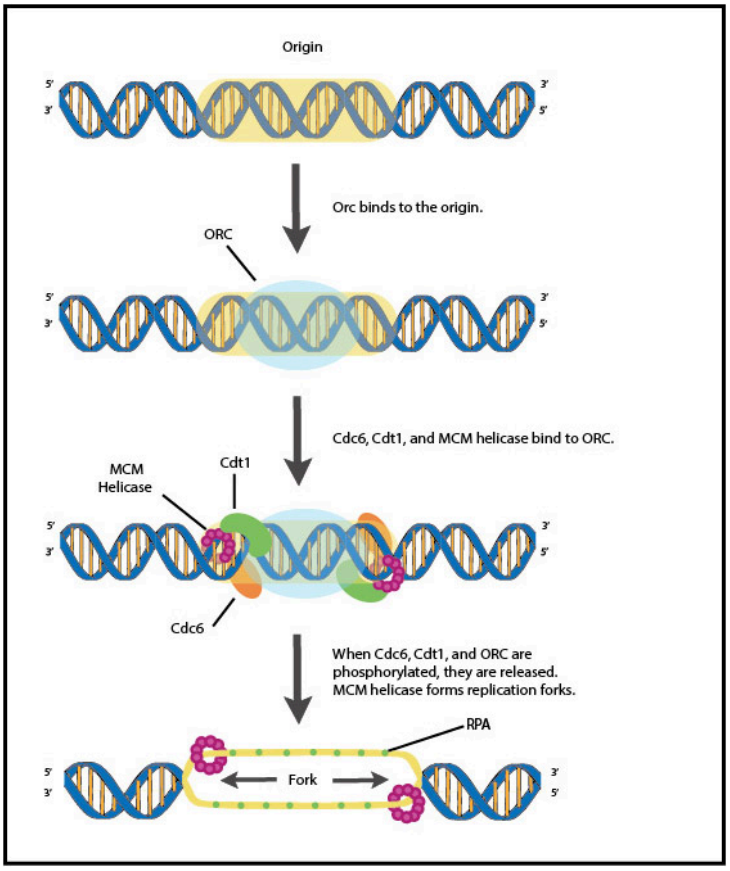

Figure 6.11 ***Replication Initiation in Eukaryotes*** *--- Image created by SL*

Key Questions

- What are the names and functions of the five proteins that participate in DNA replication initiation in eukaryotes?

Replication Elongation in Eukaryotes

MCM helicase continues DNA strand separation during the elongation phase of DNA replication, causing the replication forks to proceed in both directions away from each origin. **RPA** prevents the separated DNA strands from reforming hydrogen bonds. The separation of the DNA strands by MCM helicase generates positive supercoiling ahead of each replication fork. **Topoisomerase II** is located ahead of each replication fork and produces negative supercoiling to compensate for the positive supercoiling produced by MCM helicase. Topoisomerase II cleaves ATP to generate negative supercoils.

There are over a dozen different DNA polymerases in a typical eukaryotic cell. These eukaryotic DNA polymerases are named according to the Greek alphabet (α, β, γ, etc.). **DNA polymerases alpha (α), delta (δ)**, and **epsilon (ε)** are the DNA polymerases involved in replicating nuclear DNA in eukaryotes (see **figure 6.12**). DNA polymerase α binds to **DNA primase** to form a protein complex that synthesizes hybrid nucleic acid strands composed of 10 RNA nucleotides followed by 10–30 DNA nucleotides. These hybrid nucleic acid strands are used as primers by DNA polymerases δ and ε. DNA primase synthesizes the RNA component of the hybrid primer, while DNA polymerase α synthesizes the DNA component of the hybrid primer. Note that DNA polymerase α has both 5′ to 3′ polymerase and 3′ to 5′ exonuclease

(proofreading) activity. Once the primer is made, DNA polymerase α is released and is replaced by either DNA polymerase δ or DNA polymerase ε (i.e., the so-called **polymerase switch)**.

DNA polymerases δ and ε are the processive eukaryotic DNA polymerases. These two DNA polymerases bind to **proliferating cell nuclear antigen (PCNA)**, a protein that functions as a sliding clamp, increasing the processivity of DNA polymerases δ and ε. Once bound to PCNA, DNA polymerase ε synthesizes the leading strand, whereas the PCNA:DNA polymerase δ complex synthesizes the lagging DNA strand. Both DNA polymerases ε and δ contain 5′ to 3′ polymerase and 3′ to 5′ exonuclease (proofreading) activity. All three eukaryotic DNA polymerases (α, δ, and ε) cleave dNTPs during DNA synthesis. The released energy powers DNA replication, while the nucleoside monophosphates (dAMP, dTMP, dCMP, and dGMP) are incorporated into the growing daughter DNA strands.

Finally, **flap endonuclease** (**Fen1**) removes the RNA nucleotides of each primer, and **DNA ligase I** forms the final covalent bonds to link adjacent Okazaki fragments in the lagging DNA strands. DNA ligase I cleaves ATP during ligation.

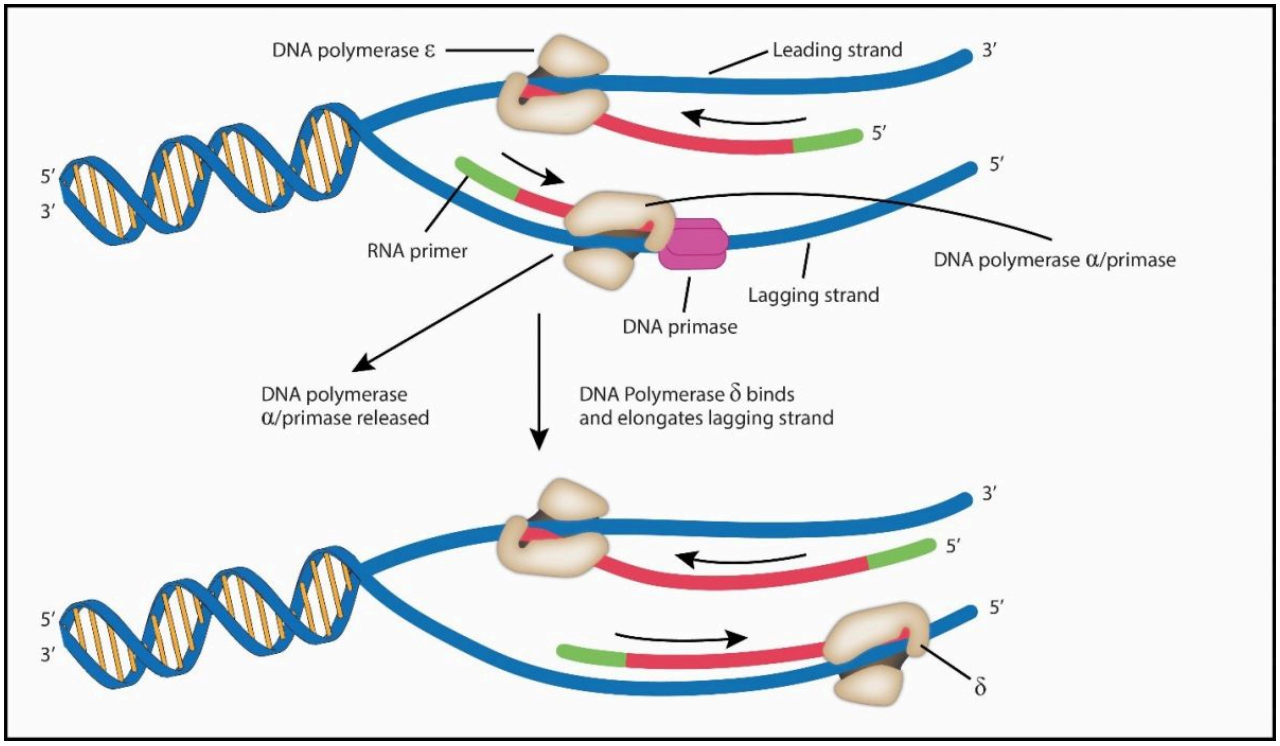

Figure 6.12 ***Replication Elongation in Eukaryotes*** *--- Image created by SL.*

Key Questions

- What are the eukaryotic equivalents of the *E. coli* enzymes DNA helicase, SSBPs, DNA gyrase, DNA primase, DNA polymerase III holoenzyme, DNA polymerase I, and DNA ligase?
- Which eukaryotic replication enzyme synthesizes the leading DNA strand?
- Which enzyme synthesizes the lagging DNA strand?
- Which eukaryotic replication elongation enzymes cleave ATP?
- Which enzymes cleave dNTPs?

Replication at Chromosome Ends

The 3’ ends of the parental DNA strands within linear eukaryotic chromosomes present a potential problem during DNA replication. Suppose a primer is made for the daughter DNA strand directly opposite the 3’ end of the parental DNA strand. Once this primer is used for DNA synthesis, the primer is removed with the hope that DNA replication will fill in the primer gap. However, DNA polymerases cannot fill in the primer gap at the end of the chromosome because DNA polymerases require a 3’-OH group to begin DNA synthesis. As a result, this primer gap is not filled in, and the newly synthesized daughter DNA strand is slightly shorter than its template DNA strand. This end replication problem would result in the progressive shortening of daughter DNA strands with each round of DNA replication. Eventually, this shortening would delete genes and have a negative effect on the phenotype of the cell.

Eukaryotes solve this potential DNA replication problem by using **telomerase** to add moderately repetitive DNA sequences to the 3’ ends of the parental DNA strands prior to DNA replication (see **figure 6.13**). Telomerase is an unusual enzyme that contains a built-in RNA component (**TERC**) and a protein component (**TERT**). Thus, telomerase is an example of a **ribonucleoprotein**. The TERC component forms hydrogen bonds with the 3’ overhang DNA sequence at the ends of the two parental DNA strands. Once bound to the 3’ end of the parental DNA strands, TERT catalyzes the synthesis of additional telomere repeat sequences using the built-in TERC component of telomerase as a template. The synthesis of additional telomere repeats by telomerase occurs in the 5’ to 3’ direction. Because telomerase synthesizes DNA in the 5' to 3' direction and requires a 3'-OH group for DNA synthesis, telomerase is considered a DNA polymerase.

Once the 3’ end of the parental DNA strand is lengthened by telomerase, DNA replication of the daughter DNA strand can occur by the synthesis of a primer opposite the repeats added by telomerase. DNA synthesis from this newly added primer occurs using the DNA polymerase δ. Finally, the primer is removed by Fen1. Since the primer for the daughter DNA strand is made opposite the telomere repeat sequences added by telomerase, the loss of the primer does not affect structural genes or the phenotype of the daughter cell.

To sum this all up, telomerase lengthens the parental DNA strands prior to DNA replication, so that the replication enzymes can make the daughter DNA strands shorter. The net result is that the overall chromosome length does not change significantly because of DNA replication.

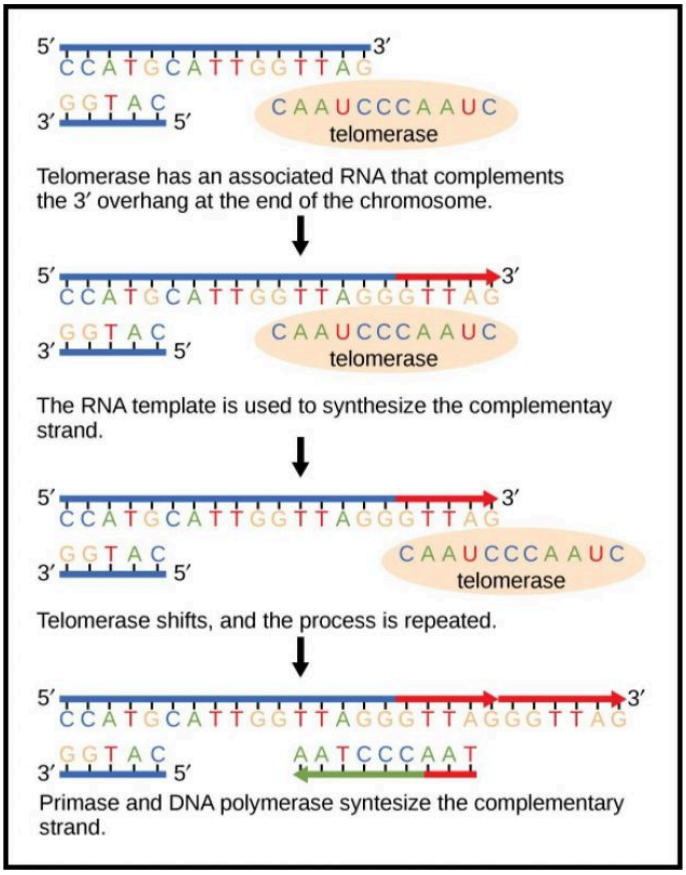

Figure 6.13 ***Telomerase Mechanism*** *--- This image is used from OpenStax (access for free at* https://openstax.org/books/biology-2e/pages/1-introduction*)*

Key Questions

- Describe the so-called "end replication problem" experienced by organisms with linear chromosomes.
- How is this end replication problem solved?
- What are the functions of the two components of telomerase?

Review Questions

Fill in the Blank:

1. The enzyme _____ methylates adenine to activate DNA replication in bacteria.
2. The enzyme _____ connects adjacent Okazaki fragments together during DNA replication in *E. coli.*
3. The _____ protein is the eukaryotic equivalent of SSBPs.
4. The enzyme _____ is composed of two subunits, called TERC and TERT.
5. During DNA replication, the template DNA strands are read by DNA polymerases in the _____ direction, while the daughter DNA strands are synthesized in the _____ direction.
6. Phosphorylation of _____ and _____ initiates DNA replication in eukaryotic organisms.
7. _____ is a eukaryotic enzyme that produces replication forks, while _____ is an *E. coli* enzyme that alleviates positive supercoiling ahead of each replication fork.
8. The _____ subunit of the DNA polymerase III holoenzyme is responsible for proofreading, while the _____ subunit is responsible for DNA synthesis.
9. _____ is an unusual DNA polymerase that contains a built-in RNA template molecule.
10. The enzyme _____ has both 5'- 3' polymerase and 5' - 3' exonuclease activity.
11. _____ binds directly to the ARS element, while _____ synthesizes the leading DNA strand in eukaryotes.