DNA Replication (The Fork)

Introduction

Replication is the process of copying the entire genetic code. Both strands are copied at the same time. We have to identify the start site of replication (the **origin**), unzip the DNA, then copy that DNA. We don't have to choose which base pairs get copied or how often, as we do in transcription, because in replication all of the nucleotides are copied. What we *will* need to focus on is how both strands get copied at the same time, even though the DNA polymerase that copies them travels in one direction only.

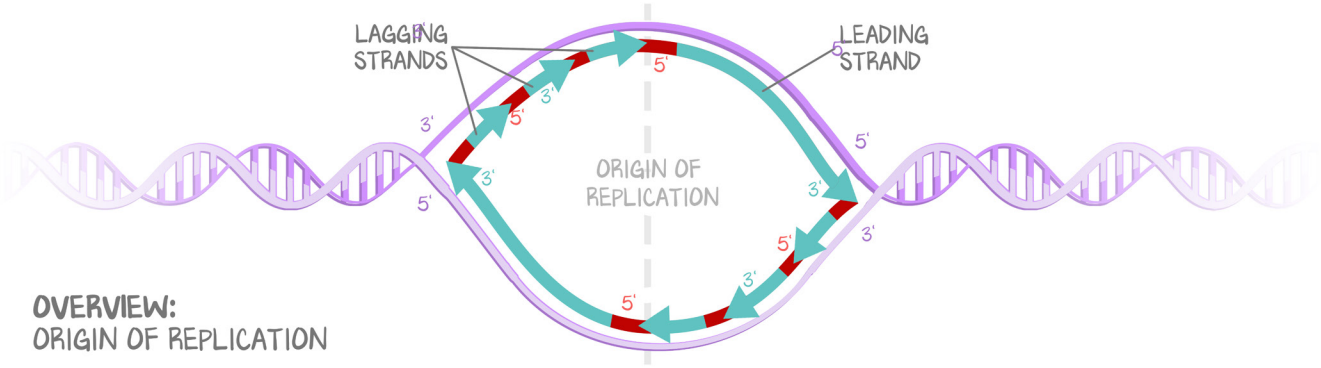

Figure 5.1: Leading and Lagging Strands
The origin of replication reveals a continuous uninterrupted leading strand traveling towards the replication fork, and a discontinuous lagging strand building towards the origin on both strands.

Even though we aren't prokaryotes, the system that is much better understood and well studied is the prokaryotic system. The enzyme names and the process we're going to discuss are in bacteria, but the principles immediately translate to human genes. We'll speak of all bacteria, then give a translating table at the end. We're discussing **prokaryotic, double-stranded DNA replication**.

Some texts use **template strand** to mean "one of the two original single strands of DNA," and others use parent strand. We'll use template. In a similar fashion, **daughter** or synthesizing strand can refer to "one of the new strands being built"; we'll use **replicated**.

Initiation

The **origin** site is identified. An enzyme named **helicase** uses ATP to **unzip the base pairs**, cleaving the hydrogen bonds between the base pairs of the two strands. This unzipping exposes the now single-stranded DNA to endonucleases which would destroy an unpaired nucleotide. To prevent damage by endonucleases and to prevent the two strands from simply **reannealing** (a fancy word that means "zipping back shut") as the helicase moves onward, **single-stranded binding proteins** bind to the single strands and stabilize them. This leaves the single-stranded DNA both safe from destruction and accessible to DNA polymerase.

RNA Priming

DNA polymerase, the enzyme that replicates DNA, can't start its own nucleotide chain; it can only add to an existing 3' end. In order for DNA polymerase to get started, an **RNA primer** is required at the origin site. **RNA primase** lays this initial primer. It starts 5' to the left of its own strand, and moves 3' to the right, which means it's starting on the 3' end of the template strand and moving towards the 5' template strand end. The RNA primer is complementary and antiparallel to the template DNA strand. The template strand is DNA (GC, AT) while the RNA primer is RNA (GC, AU). This means we will need something at the end to remove the RNA nucleotides from the copied DNA.

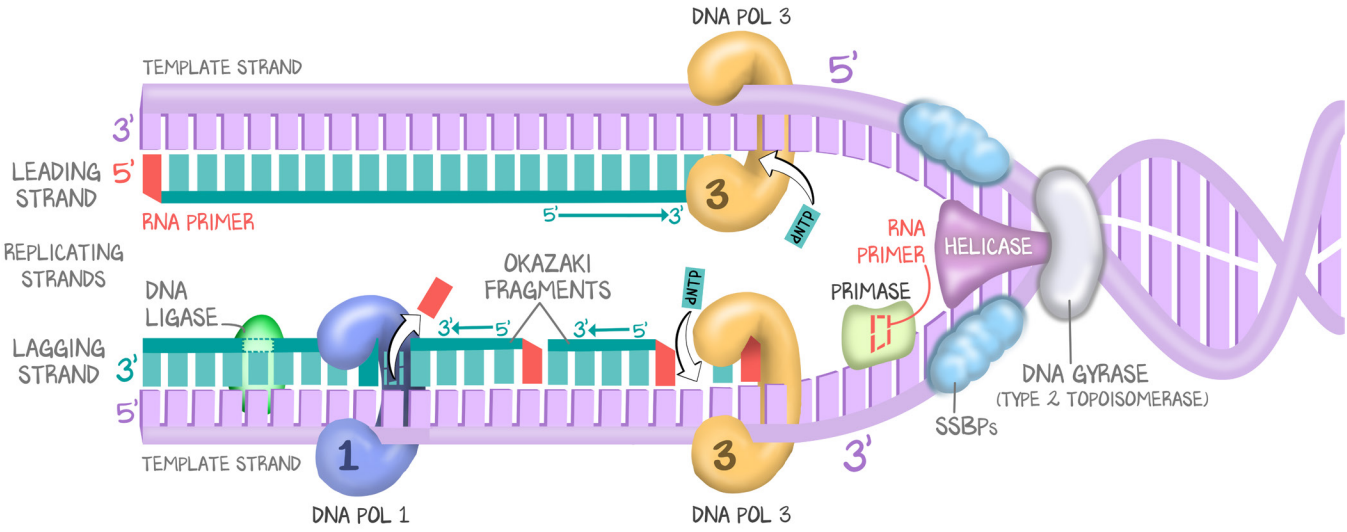

Figure 5.2: The Replication Fork
A highly detailed illustration of all the elements of replication. This one half of the replication bubble, zooming in on the right replication fork. Use this as a reference as you continue through the lesson.

DNA Polymerase

DNA polymerase III begins building its own strand 5' to the left, 3' to the right, moving from the left to the right. That means the template strand is 3' to the left, 5' to the right. DNA polymerase adds onto its own strand's 3' end, adding an incoming new nucleotide to the 3' (which has an -OH at the 3 position) by making a bond between the incoming nucleotide's 5-carbon phosphate and the strand's 3-carbon hydroxyl. Because it's moving on the page 5' left to 3' right, it's reading the template strand, which is oriented 3' left, 5' right.

The helicase enzyme continues to unzip the template strands, traveling in both directions simultaneously. DNA polymerase started where there was an RNA primer. The RNA primer began at the origin site. DNA polymerase moves towards the helicase, building the replicating strand in a **continuous fashion** into the **replication fork**. This complete, intact, continuous development is referred to as the **leading strand**.

The moving-forward-with-the-helicase-directionality is the leading strand. But both DNA polymerases are moving **away from each other**, leaving the complementary parent strand simply dangling as a single strand . . . in both directions. That would lead to catastrophic DNA damage. So while the leading strands are being built as continuous uninterrupted strands, mini-versions of the leading strand—RNA primase laying the primer so DNA polymerase can do its thing—keep starting periodically. Wherever the helicase opens up a space that RNA primase can bind, it does. Every place RNA primase binds and

lays a primer, DNA polymerase binds and starts to build a DNA strand. DNA polymerase goes in the same direction as always, starting on its own 5' left adding on moving 3' right, starting on the 3' left side of the template strand. This piecemeal replication of the **lagging strand** is synthesized discontinuously in a series of small fragments called **Okazaki fragments**.

While DNA polymerase III is adding nucleotides, it's also **proofreading** its own work. If there's an inappropriate base pair inserted into the last spot (the rightmost 3' of the growing replicating strand), then there will be abnormal bonding to the template strand. DNA polymerase has a **3' exonuclease** ability (aka it can remove the last nucleotide in the final 3' position if there was a wrong insertion). It removes the wrong one and puts the right one in.

Removal and Ligation

Every time DNA polymerase starts, there is an RNA primer. Both leading strands are started by an RNA primer. Every Okazaki fragment has an RNA primer. Every DNA polymerase heads in the same direction on the same strand, so each completed Okazaki fragment starts with an RNA primer and ends with a space. The space is not big enough for another nucleotide, but the 3' end of the DNA Okazaki fragment runs into the 5' end of the RNA primer.

RNA can't be in DNA. That means each of these RNA primers needs to be **removed**, and DNA nucleotides added in their place. In prokaryotes, **DNA polymerase I** both removes the primer (removal is by a **5' exonuclease** meaning it starts at the 5' and move towards the 3' of the copied strand) and adds DNA nucleotides to the 3' end of the Okazaki fragments.

An **exo**nuclease is able to remove nucleotides only from free-floating, end-of-strand positions. The Okazaki fragment and the RNA primer are near one another, but not connected; there isn't a phosphate bond to break. Exonuclease means that it needs a loose nucleotide; it can't break the phosphodiester bonds between nucleotides. Endonuclease means it can break the phosphodiester bond.

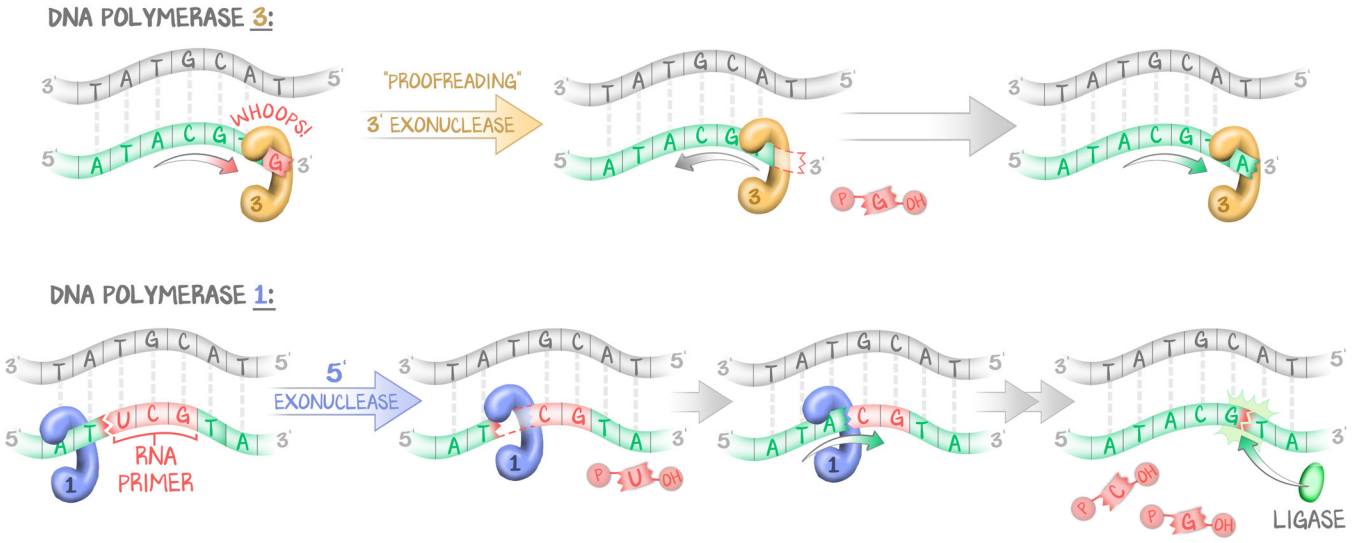

Figure 5.3: Exonuclease Activity
DNA Polymerase 3 has 3' exonuclease activity, removing a nucleotide from the 3' end. DNA polymerase I has 5' exonuclease activity, removing nucleotides from the 5' end (DNA pol 3 happens also to fill them in as it goes).

Imagine the free 3-carbon position of the last nucleotide of the Okazaki fragment right next to the 5-carbon phosphate of the first nucleotide of the RNA primer. As the DNA polymerase adds to the Okazaki fragment, it also removes the RNA nucleotide to make room for the incoming DNA nucleotide, extending the Okazaki fragment while removing the RNA primer.

Finally, with the RNA fragments removed, the gaps of DNA left by the removal of those RNA fragments filled in, all that needs happen is the small connection between Okazaki fragments get sealed by **DNA ligase**.

Supercoil

As helicase unzips the DNA, it's inherently inducing positive supercoils in the DNA ahead of it. At the replication fork, helicase opens the DNA. In front of the helicase, the not-yet-unzipped region supercoils. **DNA gyrase** (prokaryotes) and **topoisomerase** (eukaryotes) induce nicks in that leading DNA to prevent that supercoiling.

STEPS IN REPLICATION	PROKARYOTES	EUKARYOTES
Origin	One ori per circular chromosome	**Multiple** ori sites per **linear** chromosome
Unwinding of DNA	Helicase	Helicase
Single-strand stabilization	Single-stranded binding protein	Single-stranded binding protein
RNA primers	Primase	Primase
Synthesis of DNA	DNA polymerase III	**DNA polymerase α**
Removal of RNA primers (5' 3' exonuclease)	DNA polymerase I	**RNase H**
DNA gap fillers	DNA polymerase I	**DNA polymerase α**
Joining fragments	DNA ligase	DNA ligase
Supercoil hinge	DNA gyrase	**DNA topoisomerase II**
Telomeres	None	**Telomerase**

Table 5.1: Comparing Prokayotes to Eukaryotes