10.3: DNA replication

The process of DNA replication can be summarized as follows:

1. DNA unwinds at the origin of replication.
2. Helicase opens up the DNA-forming replication forks; these are extended bidirectionally.
3. Single-strand binding proteins coat the DNA around the replication fork to prevent rewinding of the DNA.
4. Topoisomerase binds at the region ahead of the replication fork to prevent supercoiling.
5. Primase synthesizes RNA primers complementary to the DNA strand.
6. DNA polymerase III starts adding nucleotides to the 3′-OH end of the primer.
7. Elongation of both the lagging and the leading strand continues.
8. RNA primers are removed by exonuclease activity.
9. Gaps are filled by DNA pol I by adding dNTPs.
10. The gap between the two DNA fragments is sealed by DNA ligase, which helps in the formation of phosphodiester bonds.

Figure 10.7: Summary of DNA replication.
DNA replication

The essential steps of replication are the same for both prokaryotes and eukaryotes. Before replication can start, the DNA has to be made available as a template. Eukaryotic DNA is bound to basic proteins known as histones to form structures called nucleosomes. Histones must be removed and then replaced during the replication process, which helps account for the lower replication rate in eukaryotes. The chromatin (the complex between DNA and proteins) may undergo some chemical modifications, so that the DNA may be able to slide off the proteins or be accessible to the enzymes of the DNA replication machinery.

One of the key players in DNA replication is the enzyme DNA polymerase, also known as DNA pol, which adds nucleotides one-by-one to the growing DNA chain that is complementary to the template strand.

In prokaryotes, three main types of polymerases are known: DNA pol I, DNA pol II, and DNA pol III.

In eukaryotes there are fourteen known polymerases, of which five are known to have major roles during replication and have been well studied. They are known as pol α, pol β, pol γ, pol δ, and pol ε.

How does the replication machinery know where to begin?

There are specific nucleotide sequences called origins of replication where replication begins. In prokaryotes, there is typically a single origin of replication on its one chromosome, and this is in contrast to eukaryotes that have many origins of replication across the chromosomes.

The origin of replication is recognized by certain proteins that bind to this site. An enzyme called helicase unwinds the DNA by breaking the hydrogen bonds between the nitrogenous base pairs. ATP hydrolysis is required for this process. As the DNA opens up, Y-shaped structures called replication forks are formed. Two replication forks are formed at the origin of replication, and these get extended bidirectionally as replication continues. Single-strand binding proteins coat the single strands of DNA near the replication fork to prevent the single-stranded DNA from winding back into a double helix.

DNA polymerase has two important restrictions. First, it is able to add nucleotides only in the 5′ to 3′ direction (a new DNA strand can be only extended in this direction). Second, it also requires a free 3′-OH group to which it can add nucleotides by forming a phosphodiester bond between the 3′-OH end and the 5′ phosphate of the next nucleotide. This essentially means that it cannot add nucleotides if a free 3′-OH group is not available.

Then how does it add the first nucleotide? The problem is solved with the help of a primer that provides the free 3′-OH end. RNA primase synthesizes an RNA segment that is about five to ten nucleotides long and complementary to the template DNA. Because this sequence primes the DNA synthesis, it is appropriately called the primer. DNA polymerase can now extend this RNA primer, adding nucleotides one-by-one that are complementary to the template strand.

The DNA tends to become more highly coiled ahead of the replication fork. Topoisomerase breaks and reforms DNA’s phosphate backbone ahead of the replication fork, thereby relieving the pressure that results from this “supercoiling.” Single-strand binding proteins bind to the single-stranded DNA to prevent the helix from re-forming.
Because DNA polymerase can only extend in the 5′ to 3′ direction, and because the DNA double helix is antiparallel, there is a problem at the replication fork. The two template DNA strands have opposing orientations: one strand is in the 5′ to 3′ direction, and the other is oriented in the 3′ to 5′ direction. Only one new DNA strand, the one that is complementary to the 3′ to 5′ parental DNA strand, can be synthesized continuously toward the replication fork. This continuously synthesized strand is known as the leading strand. The other strand, complementary to the 5′ to 3′ parental DNA, is extended away from the replication fork in small fragments known as Okazaki fragments, each requiring a primer to start the synthesis. New primer segments are laid down in the direction of the replication fork, but each pointing away from it.

The overall direction of the lagging strand will be 3′ to 5′, and that of the leading strand 5′ to 3′. A protein called the sliding clamp holds the DNA polymerase in place as it continues to add nucleotides. The sliding clamp is a ring-shaped protein that binds to the DNA and holds the polymerase in place. As synthesis continues, the RNA primers are removed by the exonuclease activity of DNA pol I, which uses DNA behind the RNA as its own primer and fills in the gaps left by removal of the RNA nucleotides by the addition of DNA nucleotides. The nicks that remain between the newly synthesized DNA (that replaced the RNA primer) and the previously synthesized DNA are sealed by the enzyme DNA ligase, which catalyzes the formation of phosphodiester linkages between the 3′-OH end of one nucleotide and the 5′ phosphate end of the other fragment.

Once the chromosome has been completely replicated, the two DNA copies move into two different cells during cell division.

**Telomere replication**

In eukaryotes, leading strand synthesis continues until the end of the chromosome is reached. On the lagging strand, DNA is synthesized in short stretches, each of which is initiated by a separate primer. When the replication fork reaches the end of the linear chromosome, there is no way to replace the primer on the 5′ end of the lagging strand.

The DNA at the ends of the chromosome thus remains unpaired, and over time these ends, called telomeres, may get progressively shorter as cells continue to divide.

Telomeres comprise repetitive sequences that code for no particular gene. In humans, a six-base-pair sequence, TTAGGG, is repeated 100 to 1,000 times in the telomere regions. In a way, these telomeres protect the genes from getting deleted as cells continue to divide. The telomeres are added to the ends of chromosomes by a separate enzyme, telomerase (figure 10.8), whose discovery helped in the understanding of how these repetitive chromosome ends are maintained. The telomerase enzyme contains a catalytic part and a built-in RNA template. It attaches to the end of the chromosome, and DNA nucleotides complementary to the RNA template are added on the 3′ end of the DNA strand. Once the 3′ end of the lagging strand template is sufficiently elongated, DNA polymerase can add the nucleotides complementary to the ends of the chromosomes. Thus, the ends of the chromosomes are replicated.
Telomerase has an associated RNA that complements the 3’ overhang at the end of the chromosome.

The RNA template is used to synthesize the complementary strand.

Telomerase shifts, and the process is repeated.

Primase and DNA polymerase synthesize the complementary strand.

Figure 10.8: Summary of telomerase activity to fill the overhang on the lagging strand.


<table>
<thead>
<tr>
<th>Prokaryotic/Eukaryotic protein</th>
<th>Specific function</th>
</tr>
</thead>
<tbody>
<tr>
<td>DNA pol I</td>
<td>Removes RNA primer and replaces it with newly synthesized DNA</td>
</tr>
<tr>
<td>DNA pol III/Pol delta and epsilon</td>
<td>Main enzyme that adds nucleotides in the 5'-3' direction</td>
</tr>
<tr>
<td>Helicase</td>
<td>Opens the DNA helix by breaking hydrogen bonds between the nitrogenous bases</td>
</tr>
<tr>
<td>Ligase</td>
<td>Seals the gaps between the Okazaki fragments to create one continuous DNA strand</td>
</tr>
<tr>
<td>Primase/Pol alpha</td>
<td>Synthesizes RNA primers needed to start replication</td>
</tr>
<tr>
<td>Sliding clamp</td>
<td>Helps to hold the DNA polymerase in place when nucleotides are being added</td>
</tr>
<tr>
<td>Topoisomerase</td>
<td>Helps relieve the strain on DNA when unwinding by causing breaks, and then resealing the DNA</td>
</tr>
<tr>
<td>Single-strand binding proteins (SSB)</td>
<td>Binds to single-stranded DNA to prevent DNA from rewinding back</td>
</tr>
</tbody>
</table>

Table 10.1: Prokaryotic DNA replication: enzymes and their function.

<table>
<thead>
<tr>
<th>Property</th>
<th>Prokaryotes</th>
<th>Eukaryotes</th>
</tr>
</thead>
<tbody>
<tr>
<td>Origin of replication</td>
<td>Single</td>
<td>Multiple</td>
</tr>
<tr>
<td>Rate of replication</td>
<td>1,000 nucleotides/s</td>
<td>50 to 100 nucleotides/s</td>
</tr>
<tr>
<td>DNA polymerase types</td>
<td>5</td>
<td>14</td>
</tr>
<tr>
<td>Telomerase</td>
<td>Not present</td>
<td>Present</td>
</tr>
<tr>
<td>RNA primer removal</td>
<td>DNA pol I</td>
<td>RNase H</td>
</tr>
<tr>
<td>Strand elongation</td>
<td>DNA pol III</td>
<td>Pol (\alpha), pol (\delta), pol (\epsilon)</td>
</tr>
<tr>
<td>Sliding clamp</td>
<td>Sliding clamp</td>
<td>PCNA</td>
</tr>
</tbody>
</table>

Table 10.2: Difference between prokaryotic and eukaryotic replication.

References and resources

Text

Clark, M. A. *Biology*, 2nd ed. Houston, TX: OpenStax College, Rice University, 2018, Chapter 14: DNA Structure and
Function.


**Figures**

Grey, Kindred, Figure 10.7 Summary of DNA replication. 2021. [https://archive.org/details/10.7_20210926](https://archive.org/details/10.7_20210926), CC BY 4.0.

Grey, Kindred, Figure 10.8 Summary of Telomerase activity to fill the overhand on the lagging strand. 2021. CC BY 4.0.