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.

Table 14.1 summarizes the enzymes involved in prokaryotic DNA replication and the functions of each.

Prokaryotic DNA Replication: Enzymes and Their Function

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

Table 14.1

Review the full process of DNA replication **here (http://openstaxcollege.org/l/replication_DNA)** .

14.5 | DNA Replication in Eukaryotes

By the end of this section, you will be able to do the following:

- Discuss the similarities and differences between DNA replication in eukaryotes and prokaryotes
- State the role of telomerase in DNA replication

Eukaryotic genomes are much more complex and larger in size than prokaryotic genomes. Eukaryotes also have a number of different linear chromosomes. The human genome has 3 billion base pairs per haploid set of chromosomes, and 6 billion base pairs are replicated during the S phase of the cell cycle. There are multiple origins of replication on each eukaryotic chromosome; humans can have up to 100,000 origins of replication across the genome. The rate of replication is approximately 100 nucleotides per second, much slower than prokaryotic replication. In yeast, which is a eukaryote, special sequences known as autonomously replicating sequences (ARS) are found on the chromosomes. These are equivalent to the origin of replication in *E. coli*.

The number of DNA polymerases in eukaryotes is much more than in prokaryotes: 14 are known, 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*.

The essential steps of replication are the same as in prokaryotes. 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 to 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. At the origin of replication, a pre-replication complex is made with other initiator proteins. Helicase and other proteins are then recruited to start the replication process (**Table 14.2**).

Difference between Prokaryotic and Eukaryotic Replication

Property	Prokaryotes	Eukaryotes
Origin of replication	Single	Multiple
Rate of replication	1000 nucleotides/s	50 to 100 nucleotides/s
DNA polymerase types	5	14
Telomerase	Not present	Present
RNA primer removal	DNA pol I	RNase H
Strand elongation	DNA pol III	Pol α, pol δ, pol ε
Sliding clamp	Sliding clamp	PCNA

Table 14.2

A helicase using the energy from ATP hydrolysis opens up the DNA helix. Replication forks are formed at each replication origin as the DNA unwinds. The opening of the double helix causes over-winding, or supercoiling, in the DNA ahead of the replication fork. These are resolved with the action of topoisomerases. Primers are formed by the enzyme primase, and using the primer, DNA pol can start synthesis. Three major DNA polymerases are then involved: α, δ and ε. DNA pol α adds a short (20 to 30 nucleotides) DNA fragment to the RNA primer on both strands, and then hands off to a second polymerase. While the leading strand is continuously synthesized by the enzyme pol *δ*, the lagging strand is synthesized by pol *ε*. A sliding clamp protein known as PCNA (proliferating cell nuclear antigen) holds the DNA pol in place so that it does not slide off the DNA. As pol δ runs into the primer RNA on the lagging strand, it displaces it from the DNA template. The displaced primer RNA is then removed by RNase H (AKA flap endonuclease) and replaced with DNA nucleotides. The Okazaki fragments in the lagging strand are joined after the replacement of the RNA primers with DNA. The gaps that remain are sealed by DNA ligase, which forms the phosphodiester bond.

Telomere replication

Unlike prokaryotic chromosomes, eukaryotic chromosomes are linear. As you've learned, the enzyme DNA pol can add nucleotides only in the 5' to 3' direction. In the 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 1000 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 14.16**), 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.

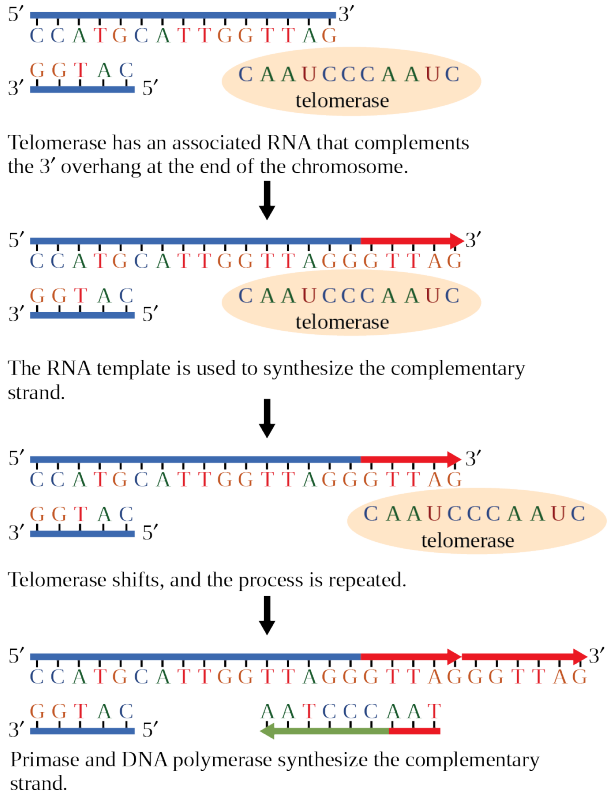

Figure 14.15 The ends of linear chromosomes are maintained by the action of the telomerase enzyme.

Telomerase is typically active in germ cells and adult stem cells. It is not active in adult somatic cells. For their discovery of telomerase and its action, Elizabeth Blackburn, Carol W. Greider, and Jack W. Szostak (**Figure 14.16**) received the Nobel Prize for Medicine and Physiology in 2009.

Figure 14.16 Elizabeth Blackburn, 2009 Nobel Laureate, is one of the scientists who discovered how telomerase works. (credit: US Embassy Sweden)

Telomerase and Aging

Cells that undergo cell division continue to have their telomeres shortened because most somatic cells do not make telomerase. This essentially means that telomere shortening is associated with aging. With the advent of modern medicine, preventative health care, and healthier lifestyles, the human life span has increased, and there is an increasing demand for people to look younger and have a better quality of life as they grow older.

In 2010, scientists found that telomerase can reverse some age-related conditions in mice. This may have potential in regenerative medicine.[2] Telomerase-deficient mice were used in these studies; these mice have tissue atrophy, stem cell depletion, organ system failure, and impaired tissue injury responses. Telomerase reactivation in these mice caused extension of telomeres, reduced DNA damage, reversed neurodegeneration, and improved the function of the testes, spleen, and intestines. Thus, telomere reactivation may have potential for treating age-related diseases in humans.

Cancer is characterized by uncontrolled cell division of abnormal cells. The cells accumulate mutations, proliferate uncontrollably, and can migrate to different parts of the body through a process called metastasis. Scientists have observed that cancerous cells have considerably shortened telomeres and that telomerase is active in these cells. Interestingly, only after the telomeres were shortened in the cancer cells did the telomerase become active. If the action of telomerase in these cells can be inhibited by drugs during cancer therapy, then the cancerous cells could potentially be stopped from further division.

14.6 | DNA Repair

By the end of this section, you will be able to do the following:

- Discuss the different types of mutations in DNA
- Explain DNA repair mechanisms

DNA replication is a highly accurate process, but mistakes can occasionally occur, such as a DNA polymerase inserting a wrong base. Uncorrected mistakes may sometimes lead to serious consequences, such as cancer. Repair mechanisms correct the mistakes. In rare cases, mistakes are not corrected, leading to mutations; in other cases, repair enzymes are themselves mutated or defective.

Most of the mistakes during DNA replication are promptly corrected by the proofreading ability of DNA polymerase itself. (**Figure 14.17**). In **proofreading**, the DNA pol reads the newly added base before adding the next one, so a correction can be made. The polymerase checks whether the newly added base has paired correctly with the base in the template strand. If it is the right base, the next nucleotide is added. If an incorrect base has been added, the enzyme makes a cut at the phosphodiester bond and releases the wrong nucleotide. This is performed by the 3' exonuclease action of DNA pol. Once the incorrect nucleotide has been removed, it

2. Jaskelioff et al., "Telomerase reactivation reverses tissue degeneration in aged telomerase-deficient mice," *Nature* 469 (2011): 102-7.