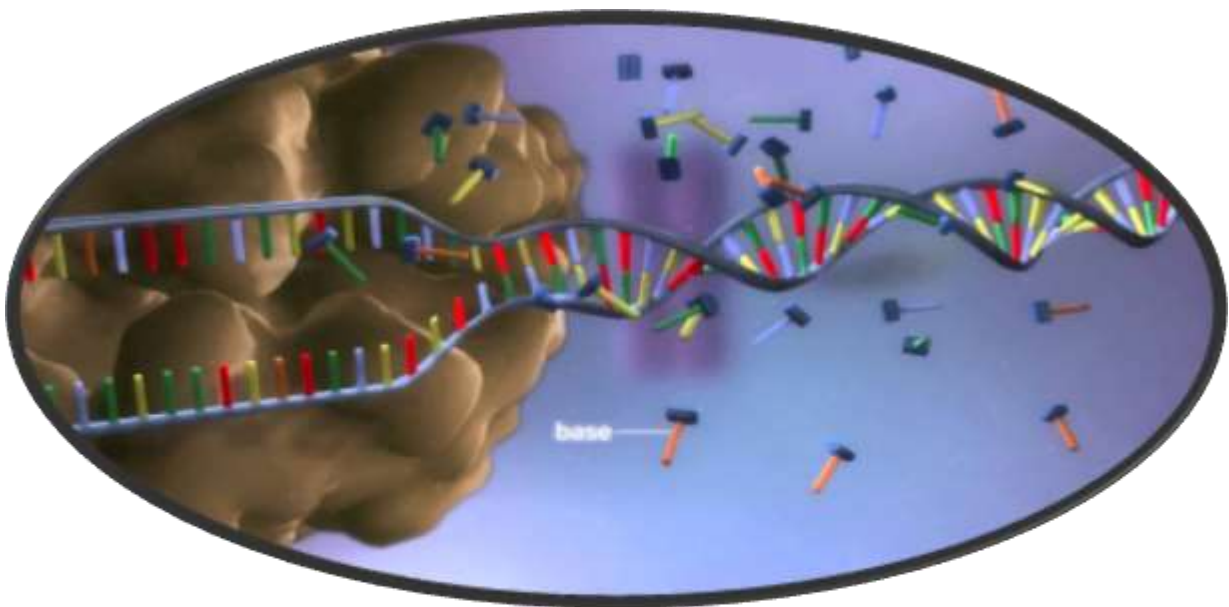




AL-Zahraa University for Women
College of Health and Medical Techniques
Anesthesia Department

DNA Replication



DNA replication

When a cell divides, it is important that **each daughter cell receives an identical copy of the DNA**. This is accomplished by the process of DNA replication. The replication of DNA occurs during the synthesis phase, or S phase, of the cell cycle, before the cell enters mitosis or meiosis.

The elucidation of the structure of the double helix provided a hint as to how DNA is copied. Recall that adenine nucleotides pair with thymine nucleotides, and cytosine with guanine. This means that the two strands are complementary to each other. For example, a strand of DNA with a nucleotide sequence of AGTCATGA will have a complementary strand with the sequence TCAGTACT ([Figure 9.8](#)).

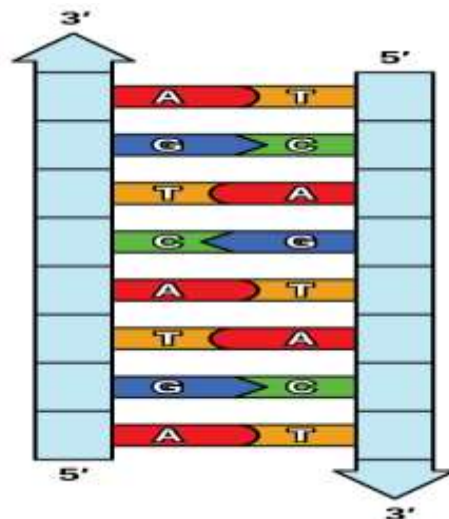


Figure 9.8 The two strands of DNA are complementary, meaning the sequence of bases in one strand can be used to create the correct sequence of bases in the other strand.

Because of the complementarity of the two strands, having one strand means that it is **possible to recreate the other strand**. This model for replication suggests that the two strands of the double helix separate during replication, and each strand serves as a template from which the new complementary strand is copied ([Figure 9.9](#)).

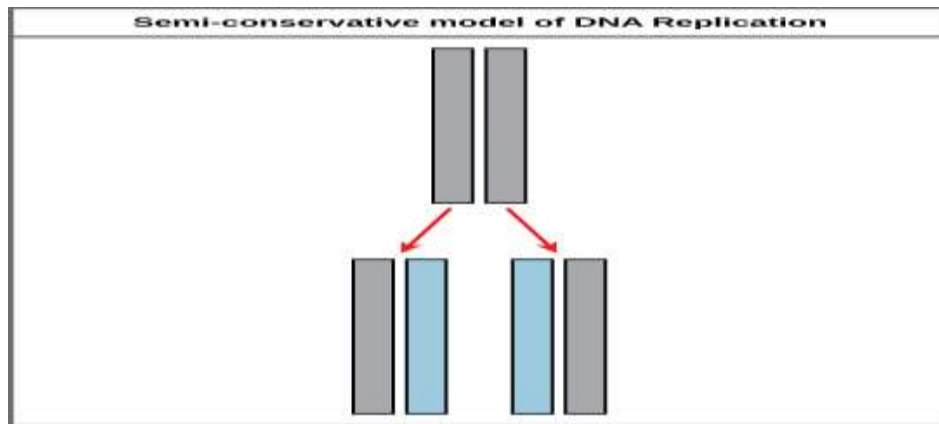


Figure 9.9 The semiconservative model of DNA replication is shown. Gray indicates the original DNA strands, and blue indicates newly synthesized DNA

During DNA replication, each of the two strands that make up the double helix serves as a template from which new strands are copied. The new strand will be complementary to the parental or “old” strand. Each new double strand consists of one parental strand and one new daughter strand. This is known as semiconservative replication. When two DNA copies are formed, they have an identical sequence of nucleotide bases and are divided equally into two daughter cells.

DNA Replication in Eukaryotes

Because eukaryotic genomes are very complex, DNA replication is a **very complicated process** that involves several enzymes and other proteins. It occurs in three main stages: initiation, elongation, and termination.

Recall that eukaryotic DNA is bound to proteins known as histones to form structures called nucleosomes. During initiation, the DNA is made accessible to the proteins and enzymes involved in the replication process. How does the replication machinery know where on the DNA double helix to begin? It turns out that there are specific nucleotide sequences called origins of replication at which replication begins. Certain proteins bind to the origin of replication while an enzyme called helicase unwinds and opens up the DNA helix. As the DNA opens up, Y-shaped structures called replication forks are formed ([Figure 9.10](#)). Two replication forks are formed at the origin of replication, and these get extended in both directions as replication proceeds. There are multiple origins of replication

on the eukaryotic chromosome, such that replication can occur simultaneously from several places in the genome.

During elongation, an enzyme called **DNA polymerase** adds DNA nucleotides to the 3' end of the template. Because DNA polymerase can only add new nucleotides at the end of a backbone, a primer sequence, which provides this starting point, is added with complementary RNA nucleotides. This primer is removed later, and the nucleotides are replaced with DNA nucleotides. One strand, which is complementary to the parental DNA strand, is synthesized continuously toward the replication fork so the polymerase can add nucleotides in this direction. This continuously synthesized strand is known as the leading strand. Because DNA polymerase can only synthesize DNA in a 5' to 3' direction, the other new strand is put together in short pieces called Okazaki fragments. The Okazaki fragments each require a primer made of RNA to start the synthesis. The strand with the Okazaki fragments is known as the lagging strand. As synthesis proceeds, an enzyme removes the RNA primer, which is then replaced with DNA nucleotides, and the gaps between fragments are sealed by an enzyme called DNA ligase.

The process of DNA replication can be summarized as follows:

1. DNA unwinds at the origin of replication.
2. New bases are added to the complementary parental strands. One new strand is made continuously, while the other strand is made in pieces.
3. Primers are removed, new DNA nucleotides are put in place of the primers and the backbone is sealed by DNA ligase

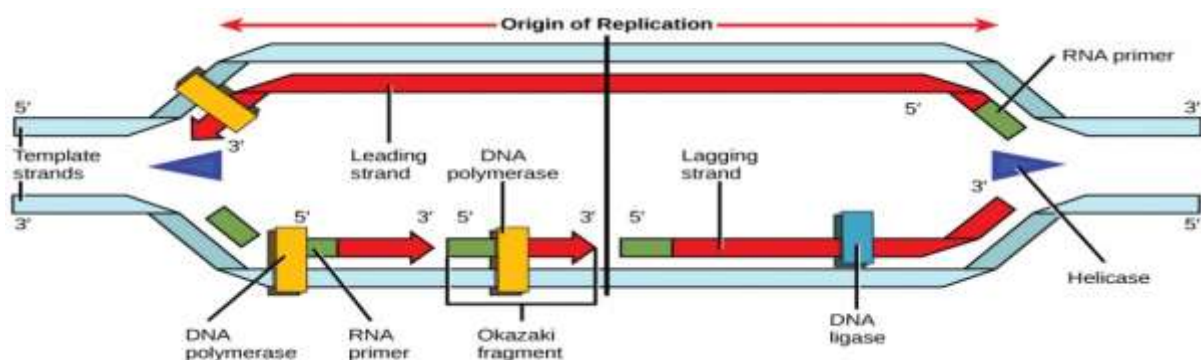


Figure 9.10 A replication fork is formed by the opening of the origin of replication, and helicase separates the DNA strands. An RNA primer is synthesized, and is elongated by the DNA polymerase. On the leading strand, DNA is synthesized continuously, whereas

on the lagging strand, DNA is synthesized in short stretches. The DNA fragments are joined by DNA ligase (not shown)

You isolate a cell strain in which the joining together of Okazaki fragments is impaired and suspect that a mutation has occurred in an enzyme found at the replication fork. Which enzyme is most likely to be mutated?

Telomere Replication

Because eukaryotic chromosomes are linear, DNA replication comes to the end of a line in eukaryotic chromosomes. As you have learned, the DNA polymerase enzyme can add nucleotides in only one direction. In the leading strand, synthesis continues until the end of the chromosome is reached; however, on the lagging strand there is no place for a primer to be made for the DNA fragment to be copied at the end of the chromosome. This presents a problem for the cell because the ends remain unpaired, and over time these ends get progressively shorter as cells continue to divide. The ends of the linear chromosomes are known as telomeres, which have repetitive sequences that do not code for a particular gene. As a consequence, it is telomeres that are shortened with each round of DNA replication instead of genes. For example, in humans, a six base-pair sequence, TTAGGG, is repeated 100 to 1000 times. The discovery of the enzyme telomerase ([Figure 9.11](#)) helped in the understanding of how chromosome ends are maintained. The telomerase attaches to the end of the chromosome, and complementary bases to the RNA template are added on the end of the DNA strand. Once the lagging strand template is sufficiently elongated, DNA polymerase can now add nucleotides that are complementary to the ends of the chromosomes. Thus, the ends of the chromosomes are replicated.

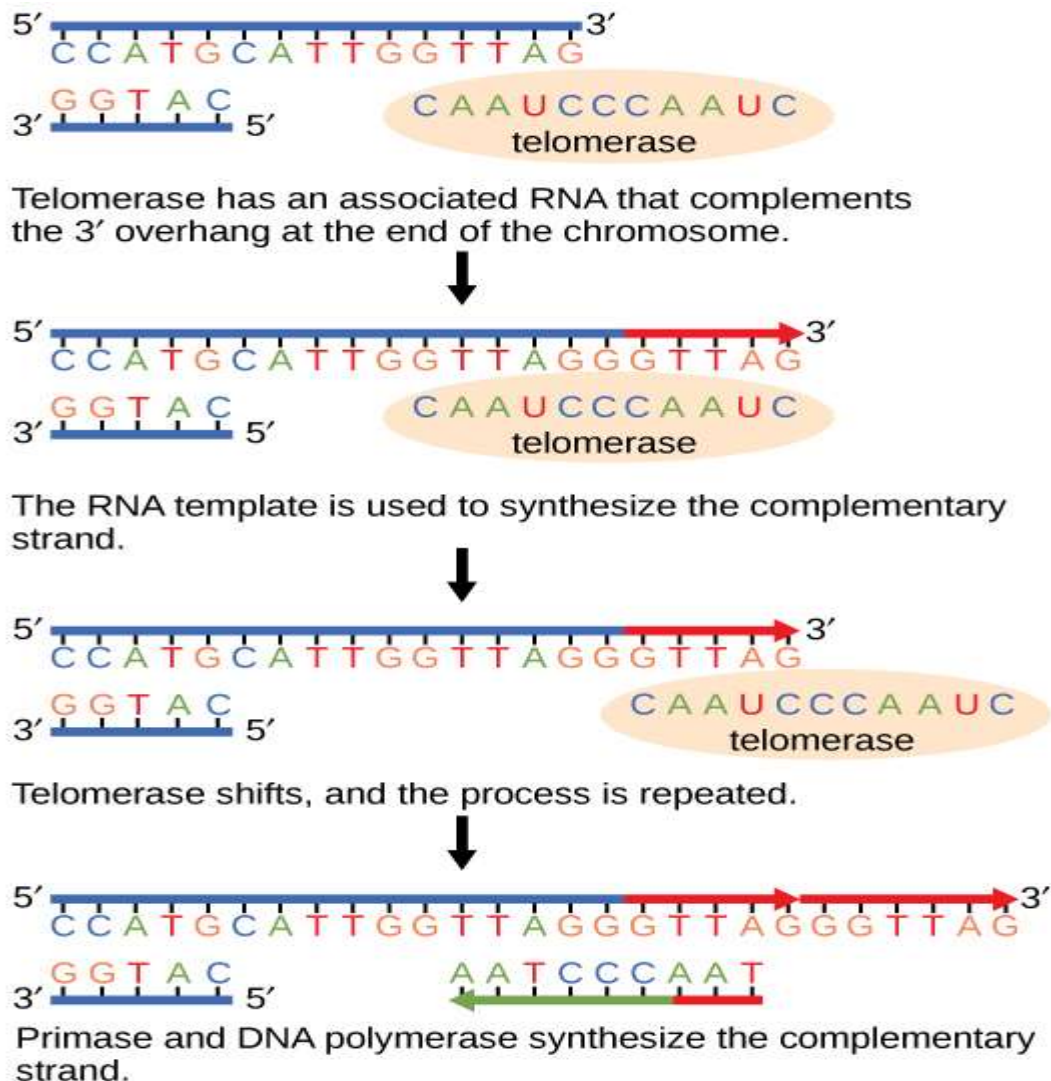


Figure 9.11 The ends of linear chromosomes are maintained by the action of the telomerase enzyme.

Telomerase is typically found to be active in germ cells, adult stem cells, and some cancer cells. For her discovery of telomerase and its action, Elizabeth Blackburn ([Figure 9.12](#)) received the Nobel Prize for Medicine and Physiology in 2009.

Telomerase is not active in adult somatic cells. Adult somatic cells that undergo cell division continue to have their telomeres shortened. This essentially means that telomere shortening is associated with aging. In 2010, scientists found that telomerase can reverse some age-related conditions in mice, and this may have potential in regenerative medicine.¹ 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 functioning of the testes, spleen, and intestines. Thus, telomere reactivation may have potential for treating age-related diseases in humans.

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Differences between Prokaryotic and Eukaryotic Replications

| Property | Prokaryotes | Eukaryotes |
|-----------------------|--------------------|-------------------------|
| Origin of replication | Single | Multiple |
| Rate of replication | 1000 nucleotides/s | 50 to 100 nucleotides/s |
| Chromosome structure | circular | linear |
| Telomerase | Not present | Present |

DNA Repair

DNA polymerase can make mistakes while adding nucleotides. It edits the DNA by proofreading every newly added base. Incorrect bases are removed and replaced by the correct base, and then polymerization continues ([Figure 9.13 a](#)). Most mistakes are corrected during replication, although when this does not happen, the mismatch repair mechanism is employed. Mismatch repair enzymes recognize the wrongly incorporated base and excise it from the DNA, replacing it with the correct base ([Figure 9.13 b](#)). In yet another type of repair, nucleotide excision repair, the DNA double strand is unwound and separated, the incorrect bases are removed along with a few bases on the 5' and 3' end, and these are replaced by copying the template with the help of DNA polymerase ([Figure 9.13 c](#)). Nucleotide excision repair is particularly important in correcting thymine dimers, which are primarily caused by ultraviolet light. In a thymine dimer, two thymine nucleotides adjacent to each other on one strand are covalently bonded to each other rather than their complementary bases. If the dimer is not removed and repaired it will lead to a mutation. Individuals with flaws in their nucleotide excision repair genes show extreme sensitivity to sunlight and develop skin cancers early in life.

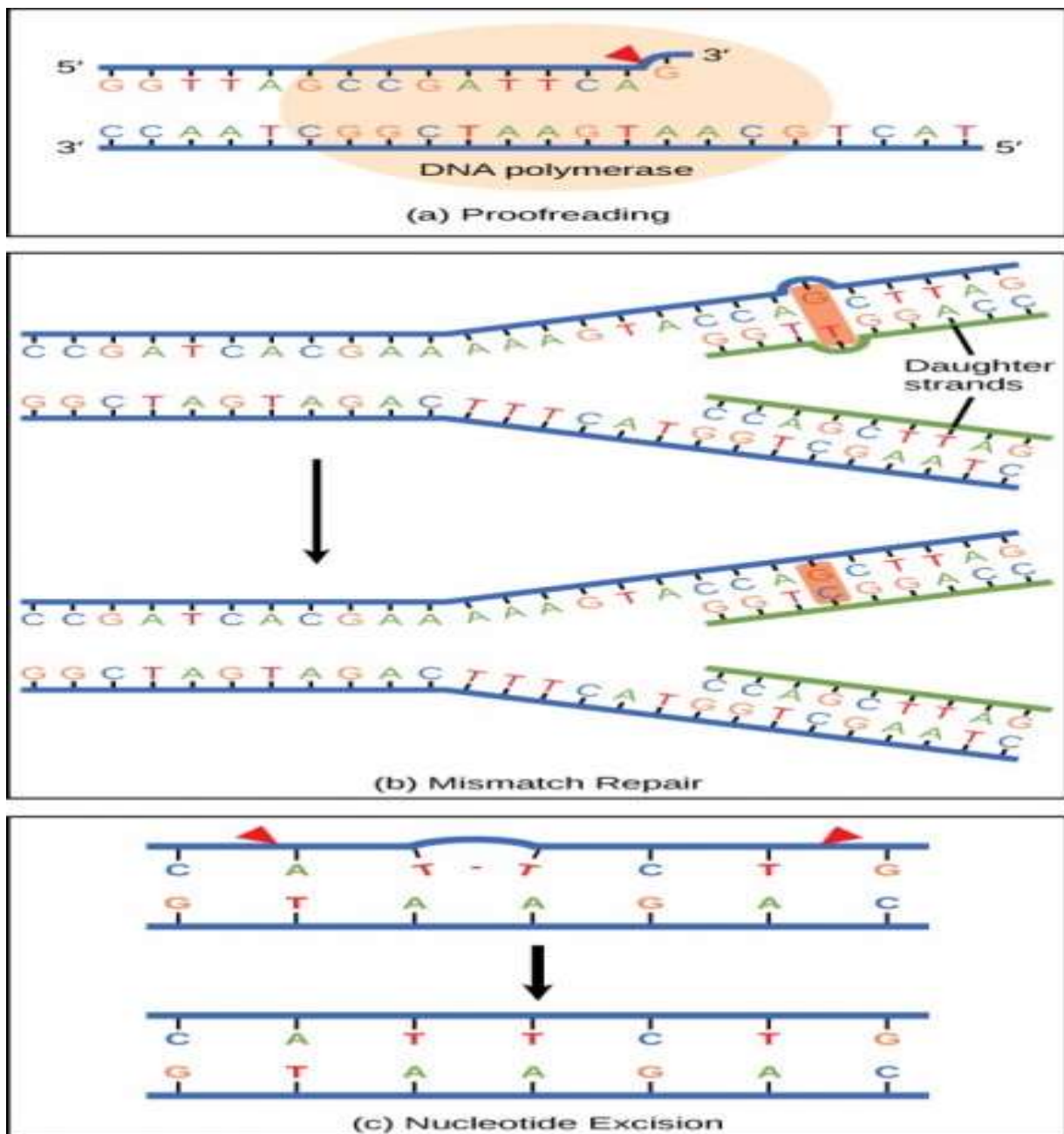


Figure 9.13 Proofreading by DNA polymerase (a) corrects errors during replication. In mismatch repair (b), the incorrectly added base is detected after replication. The mismatch repair proteins detect this base and remove it from the newly synthesized strand by nuclease action. The gap is now filled with the correctly paired base. Nucleotide excision (c) repairs thymine dimers. When exposed to UV, thymines lying adjacent to each other can form thymine dimers. In normal cells, they are excised and replaced.

Glossary

DNA ligase: the enzyme that catalyzes the joining of DNA fragments together

DNA polymerase: an enzyme that synthesizes a new strand of DNA complementary to a template strand

helicase: an enzyme that helps to open up the DNA helix during DNA replication by breaking the hydrogen bonds

lagging strand: during replication of the 3' to 5' strand, the strand that is replicated in short fragments and away from the replication fork

leading strand: the strand that is synthesized continuously in the 5' to 3' direction that is synthesized in the direction of the replication fork

mismatch repair: a form of DNA repair in which non-complementary nucleotides are recognized, excised, and replaced with correct nucleotides

mutation: a permanent variation in the nucleotide sequence of a genome

nucleotide excision repair: a form of DNA repair in which the DNA molecule is unwound and separated in the region of the nucleotide damage, the damaged nucleotides are removed and replaced with new nucleotides using the complementary strand, and the DNA strand is resealed and allowed to rejoin its complement

Okazaki fragments: the DNA fragments that are synthesized in short stretches on the lagging strand

primer: a short stretch of RNA nucleotides that is required to initiate replication and allow DNA polymerase to bind and begin replication

replication fork: the Y-shaped structure formed during the initiation of replication

semiconservative replication: the method used to replicate DNA in which the double-stranded molecule is separated and each strand acts as a template for a new strand to be synthesized, so the resulting DNA molecules are composed of one new strand of nucleotides and one old strand of nucleotides

telomerase: an enzyme that contains a catalytic part and an inbuilt RNA template; it functions to maintain telomeres at chromosome ends

telomere: the DNA at the end of linear chromosomes