Recall that eukaryotic DNA is wound around histone proteins that then coil and form structures called nucleosomes. During initiation, DNA must be unwound in order to make it accessible to binding proteins and enzymes necessary for DNA replication. 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 where replication begins. Replication binding proteins attach to the origin of replication and an enzyme called **helicase** unwinds and opens the DNA helix (Figure 10.14). As the DNA double-helix opens, Y-shaped structures called **replication forks** are formed (Figure 10.14). Two replication forks are formed at the origin of replication, and these extend in both directions. There are multiple origins of replication on eukaryotic chromosomes. This allows replication to occur simultaneously from several places within the genome.

Once initiation has occurred with the help of helicase, the DNA is now accessible. The next step of DNA replication, elongation, can now occur.

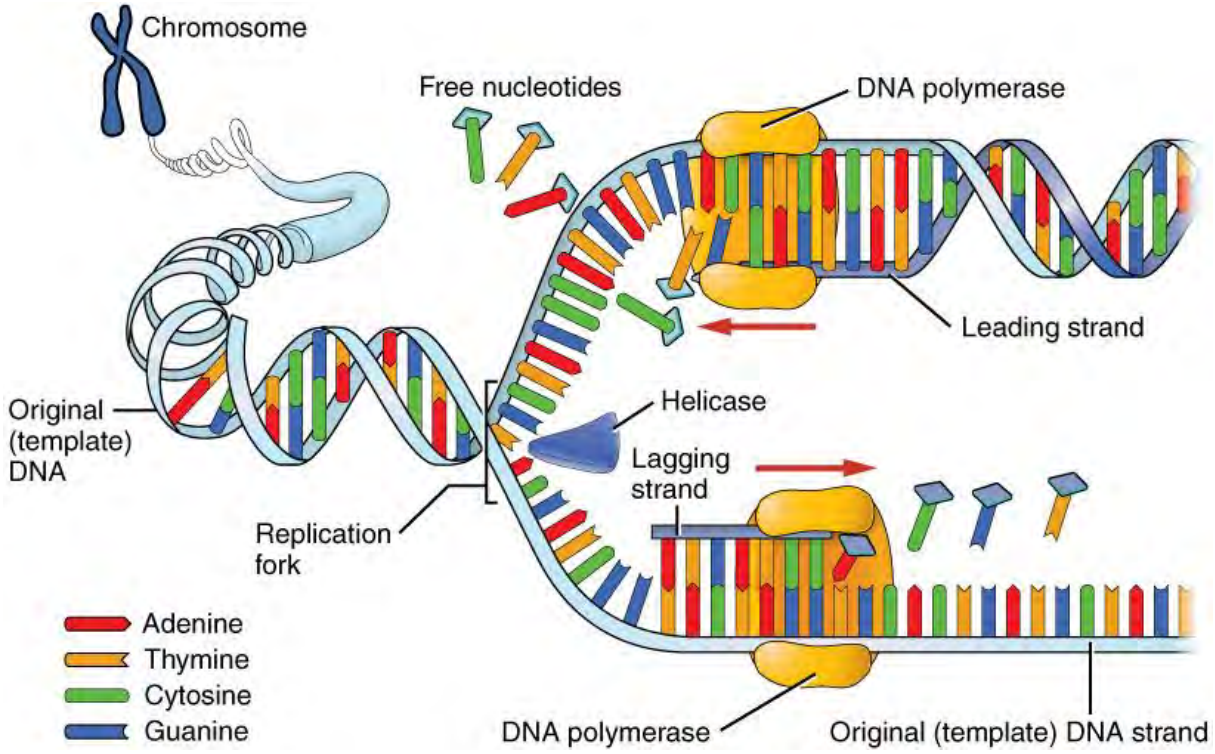

Figure 10.15 In DNA replication, DNA polymerase adds complementary base pairs. (credit: Betts et al. / Anatomy and Physiology OpenStax)

During elongation, an enzyme called **DNA polymerase** adds nucleotides one-by-one to the growing DNA strand which is complementary to the "old" parent template strand (Figure 10.15). DNA polymerase has two important restrictions. First, DNA polymerase can only add nucleotides in the 5' to 3' direction. This means, new DNA strands can only be extended or made in the 5' to 3' direction (Figure 10.14). Second, DNA polymerase requires a free 3'-OH group to which it can add nucleotides. Where does the free 3'-OH group come from? An enzyme called **RNA primase** adds a small five to ten nucleotide RNA segments, which provides the necessary

free 3'-OH end. Because this RNA sequence primes the DNA synthesis, it is appropriately called the **RNA primer**. DNA polymerase can now extend the RNA primer, adding nucleotides one-by-one that are complementary to the template strand. This primer is later removed, and the RNA nucleotides are replaced with DNA nucleotides.

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 (Figure 10.10 and 10.14). Only one new DNA strand, the one that is complementary to the 3' to 5' parental DNA strand, can be synthesized continuously towards the replication fork. This continuously synthesized strand is known as the **leading strand** (Figure 10.15). 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 Okazaki fragment requires an RNA primer to start the DNA synthesis (Figure 10.14). Okazaki fragments are named after the Japanese scientists, Tsuneko and Reiji Okazaki, who first discovered them. The strand with the Okazaki fragments is known as the **lagging strand**. As synthesis proceeds, each RNA primer is removed and replaced with DNA nucleotides. Gaps between the Okazaki fragments are filled in and sealed by an enzyme called **DNA ligase**. Termination is said to have occurred when each of the two original strands are bound to their own, finished, complementary strands.

The process of DNA replication can be summarized as follows:

1. DNA unwinds at the origin of replication with the help of specialized binding proteins.
2. Helicase opens up the DNA, forming replication forks. Each replication fork is extended in one direction.
3. RNA Primase synthesizes RNA primers complementary to the DNA strand.
4. DNA polymerase adds new nucleotides complementary to the DNA strand. The leading strand is made continuously, while the lagging strand is made in segments called Okazaki fragments.
5. RNA primers are removed, new DNA nucleotides are put in place of the RNA primers and the backbone is sealed by DNA ligase.

Check your knowledge

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?

Answer: ligase

CONCEPTS IN ACTION – Observe DNA replication in this video.

Telomere Replication

As you have learned, the DNA polymerase 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, once the end of the chromosome is reached there is no place for a RNA primer to be added. 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**. Telomeres have repetitive sequences that do not code for a gene. They are important because they prevent chromosomes from arbitrarily fusing with one another and protect the DNA from becoming damaged.

It is the 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 10.16) helped explain how chromosome ends are maintained. The **telomerase** carries its own RNA primer which can base pair to the end of the DNA strand. The telomerase can then add DNA nucleotides to the end of the chromosome, elongating it. Once the template strand is sufficiently elongated, DNA polymerase can then add nucleotides that are complementary to the ends of the chromosomes. Thus, the ends of the chromosomes are maintained in germline cells, adult stem cells, and some cancer cells.

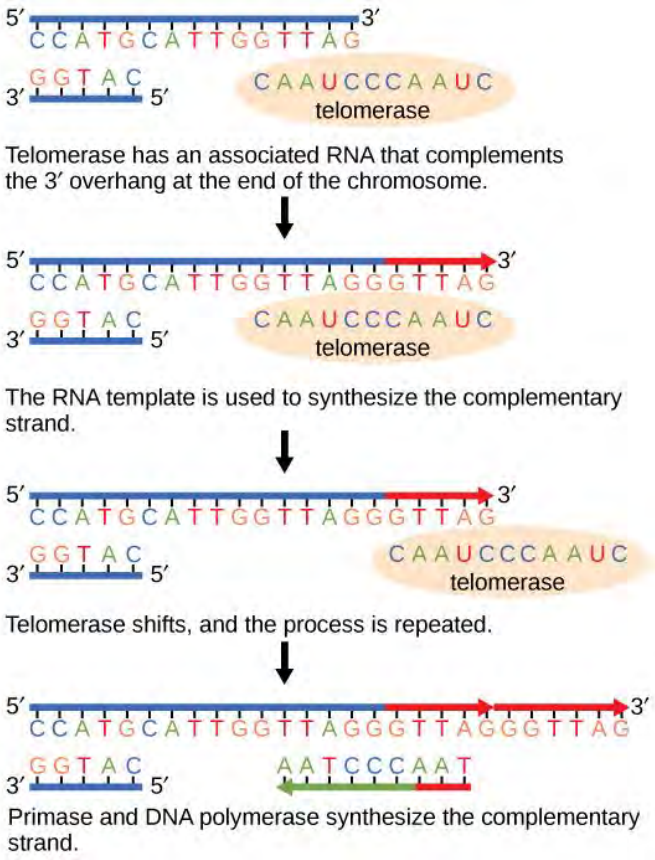

Figure 10.16 The ends of linear chromosomes are maintained by the action of the telomerase enzyme. (credit: Fowler et al. / Concepts of Biology OpenStax)