SECTION 2. DNA REPLICATION

LEARNING OBJECTIVES

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

- Describe the process of DNA replication and the functions of the enzymes involved
- Identify the differences between DNA replication in bacteria and eukaryotes

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Cells must replicate their DNA before they can divide. This process ensures that each daughter cell gets a copy of the genome, and therefore, successful inheritance of genetic traits. The basic mechanism of DNA replication is conserved in all organisms, but variations occur between prokaryotes and eukaryotes.

DNA replication is a highly sophisticated, highly coordinated series of molecular events involving many different proteins. Replication events are divided into four major stages: initiation, unwinding, primer synthesis, and elongation.

PROKARYOTIC REPLICATION

Replication commences at a region in the prokaryotic genome known as *oriC*, or origin of replication. The origin of replication is approximately 245 base pairs long and is rich in adenine-thymine (AT) sequences. A number of proteins bind at the oriC. These include:

1. DnaA - binds to the origin of replication and initiates opening of the DNA.
2. DnaB - hexameric helicase, hydrolyzes ATP to unwind the DNA double helix, forming single stranded (ss) DNA to be used as a template. The protein is ring shaped and 'clamped' on to the DNA.
3. SSBs – single-strand binding proteins coat the now single – stranded DNA to prevent re-annealing with their complementary strand
4. DNA gyrase – untwists the DNA in front of the replication fork.

A replication bubble then opens up, and replication proceeds along the two single strands at the same time but in opposite directions. This means that there are two replication forks that move along the DNA.

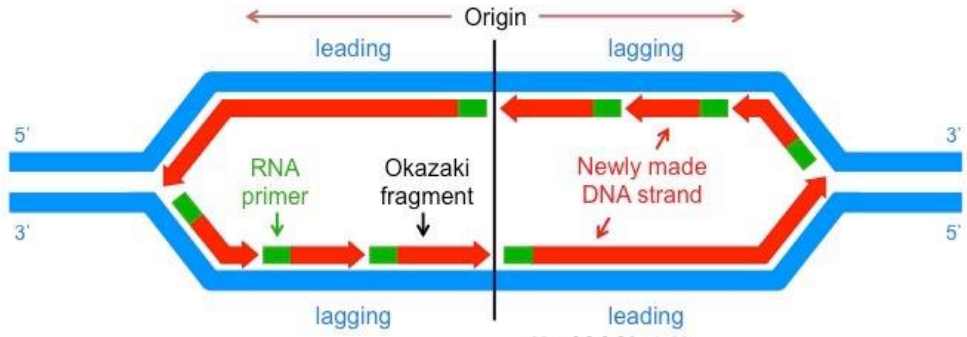

Figure 1. DNA synthesis at an origin of replication. From https://ib.bioninja.com.au/higher-level/topic-7-nucleic-acids/71-dna-structure-and-replic/origins-of-replication.html

The unwinding of DNA during replication may cause the formation of supercoils due to overwinding ahead of the replication fork. Topoisomerases bind to the double helix and relieve torsional stresses. There are two types of topoisomerases in bacteria:

1. Type I topisomerase
   - produces single – strand breaks in DNA
   - relaxes overwound, or supercoiled DNA, which arises when DNA is continuously being separated as the replication fork progresses
   - does not require energy
2. Type II topoisomerase
   - produces transient double – strand breaks
   - adds to the underwinding of DNA by introducing negative supercoils
   - requires energy in the form of ATP
   - used to separate the intertwined new daughter DNA circles at the end of bacterial replication

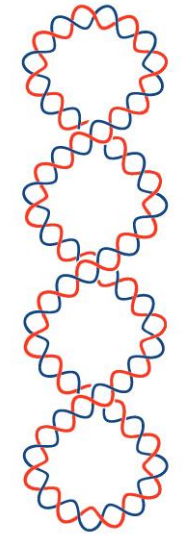

Fig. 2. Negatively supercoiled circular DNA. From https://commons.wikimedia.org/wiki/File:Negative.jpg

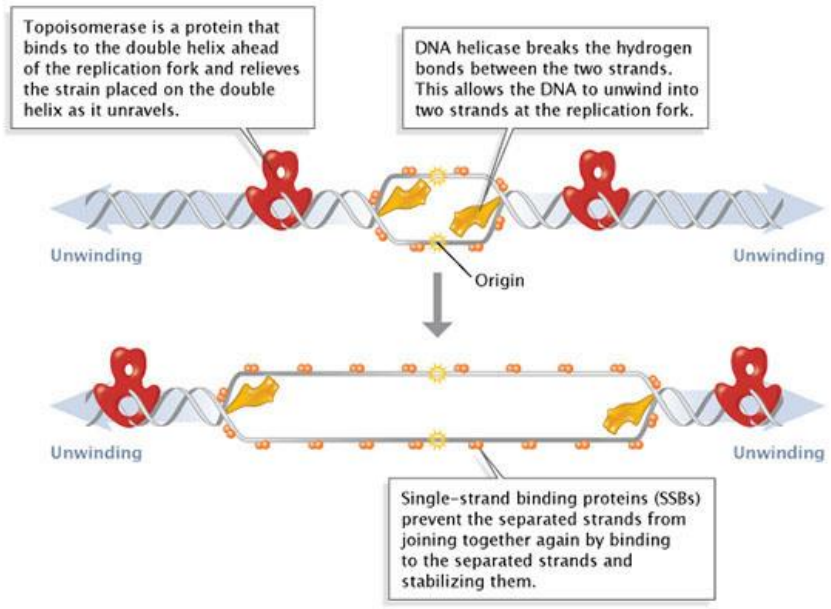

Figure 3: Facilitation of DNA unwinding.
During DNA replication, several proteins facilitate the unwinding of the DNA double helix into two single strands. Topoisomerases (red) reduce torsional strain caused by the unwinding of the DNA double helix; DNA helicase (yellow) breaks hydrogen bonds between complementary base-pairs; single-strand binding proteins (SSBs) stabilize the separated strands and prevent them from rejoining.
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Table 1. The enzymes and proteins involved in bacterial DNA replication.

Enzyme or Factor	Function
DNA pol I	Exonuclease activity removes RNA primer and replaces it with newly synthesized DNA
DNA pol III	Main enzyme that adds nucleotides in the 5' to 3' direction
Helicase	Opens the DNA helix by breaking hydrogen bonds between the nitrogenous bases
Ligase	Seals the gaps between the Okazaki fragments on the lagging strand to create one continuous DNA strand
Primase	Synthesizes RNA primers needed to start replication
Single-stranded binding proteins	Bind to single-stranded DNA to prevent hydrogen bonding between DNA strands, reforming double-stranded DNA
Sliding clamp	Helps hold DNA pol III in place when nucleotides are being added
Topoisomerase II (DNA gyrase)	Relaxes supercoiled chromosome to make DNA more accessible for the initiation of replication; helps relieve the stress on DNA when unwinding, by causing breaks and then resealing the DNA
Topoisomerase IV	Introduces single-stranded break into concatenated chromosomes to release them from each other, and then reseals the DNA

Primer synthesis marks the beginning of the actual synthesis of the new DNA molecule. Primers are short stretches of nucleotides (~10 – 12 bases long) synthesized by the enzyme primase, which is an RNA polymerase. Primers are required in replication because DNA polymerases, the enzymes responsible for the actual addition of nucleotides to the new DNA strand, can only add dNTPs to the 3'-OH group of an existing chain. The primer chain synthesized by primase provides this functional group. Later, after elongation is complete, the primer is removed and replaced with DNA nucleotides.

Elongation, or the addition of nucleotides to the new DNA strand, begins after the primer has been added. Synthesis of new DNA involves adding nucleotides according to complementarity with the template strand. Recall that one of the key features of the Watson-Crick DNA model is that adenine is always paired with thymine and cytosine is always paired with guanine. So, for example, if the original strand reads A-G-C-T, the new strand will read T-C-G-A.

The main enzyme involved in DNA synthesis is DNA polymerase. It can synthesize DNA in the 5'-to-3' direction, meaning nucleotides are added only to the 3' end of the growing strand. As shown in Figure 2, the 5'-phosphate group of the new nucleotide binds to the 3'-OH group of the last nucleotide of the growing strand. This means that synthesis and elongation can proceed in only one direction. DNA polymerase however will only add a dNMP; the two outermost phosphates of the incoming nucleotide will be released. The cleavage of the high-energy phosphate groups drives the replication process forward.

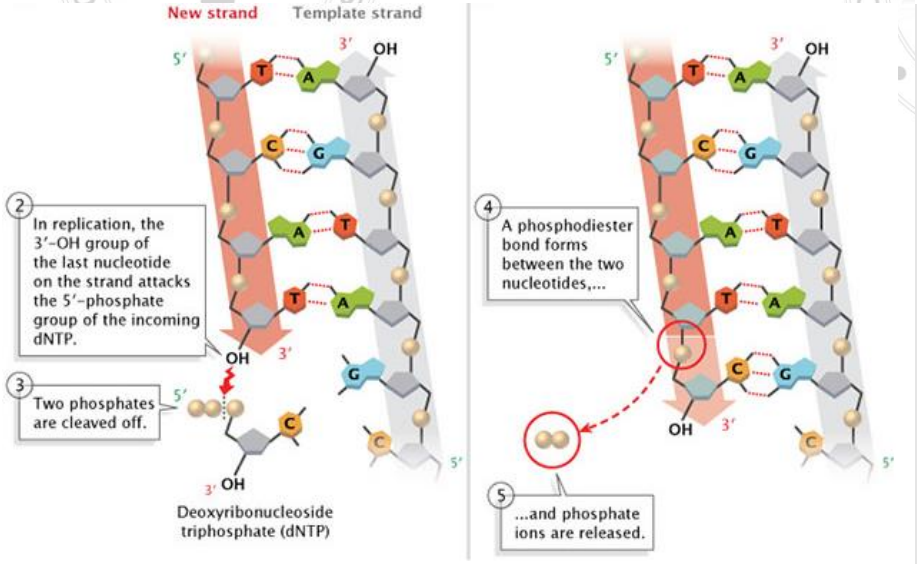

Figure 4: New DNA is synthesized from deoxyribonucleoside triphosphates (dNTPs).

(A) During DNA replication, the 3'-OH group of the last nucleotide on the new strand attacks the 5'-phosphate group of the incoming dNTP. Two phosphates are cleaved off. (B) A phosphodiester bond forms between the two nucleotides, and phosphate ions are released. **© 2014 Nature Education Adapted from Pierce, Benjamin. *Genetics: A Conceptual Approach*, 2nd ed.**

DNA polymerase can only add to the 3' end, so the 5' end of the primer remains unaltered. Because the two unwound template strands are antiparallel, replication occurs differently for each.

- **continuous replication** for the leading strand. Addition of new nucleotides can occur unimpeded as DNA polymerase moves in the same direction as the replication fork.

- **discontinuous replication** for the lagging strand. Since the 5’ to 3’ direction for this strand is moving away from the replication fork, the double helix has to unwind a bit before another primer can be formed and used to start another stretch of DNA. As a result, replication along the lagging strand can only proceed in short, discontinuous spurts (Figure 3).

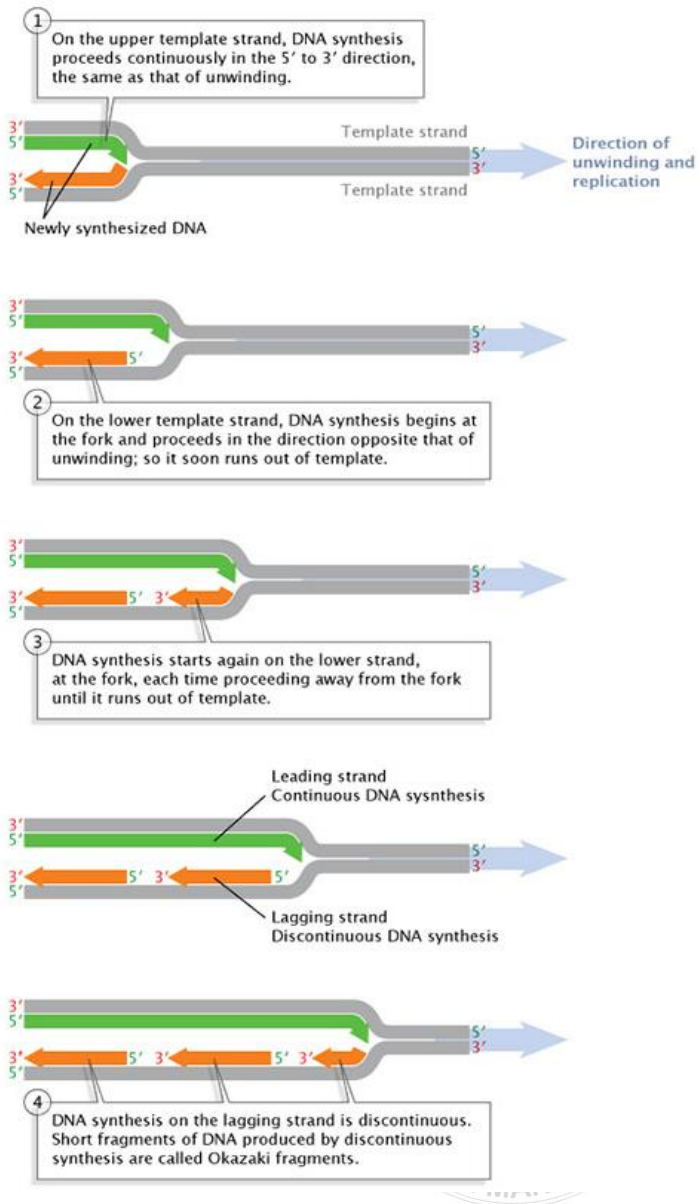

Figure 5: Replication of the leading DNA strand is continuous, while replication along the lagging strand is discontinuous.
After a short length of the DNA has been unwound, synthesis must proceed in the 5' to 3' direction; that is, in the direction opposite that of the unwinding.**© 2014 Nature Education** **Adapted from Pierce, Benjamin. *Genetics: A Conceptual Approach*, 2nd ed.**

Lagging strand synthesis produces short (600-1000 nucleotide) segments called Okazaki fragments. DNA polymerase will stop adding to the the newly formed Okazaki fragment once it reaches the next primer. DNA polymerase I and RNase H are responsible for removing the RNA primer, replacing it with DNA nucleotides. When these enzymes finish, they leave a nick between the section of DNA that was formerly the primer and the elongated section of DNA. Another enzyme called DNA ligase then acts to seal the bond between the two adjacent nucleotides.

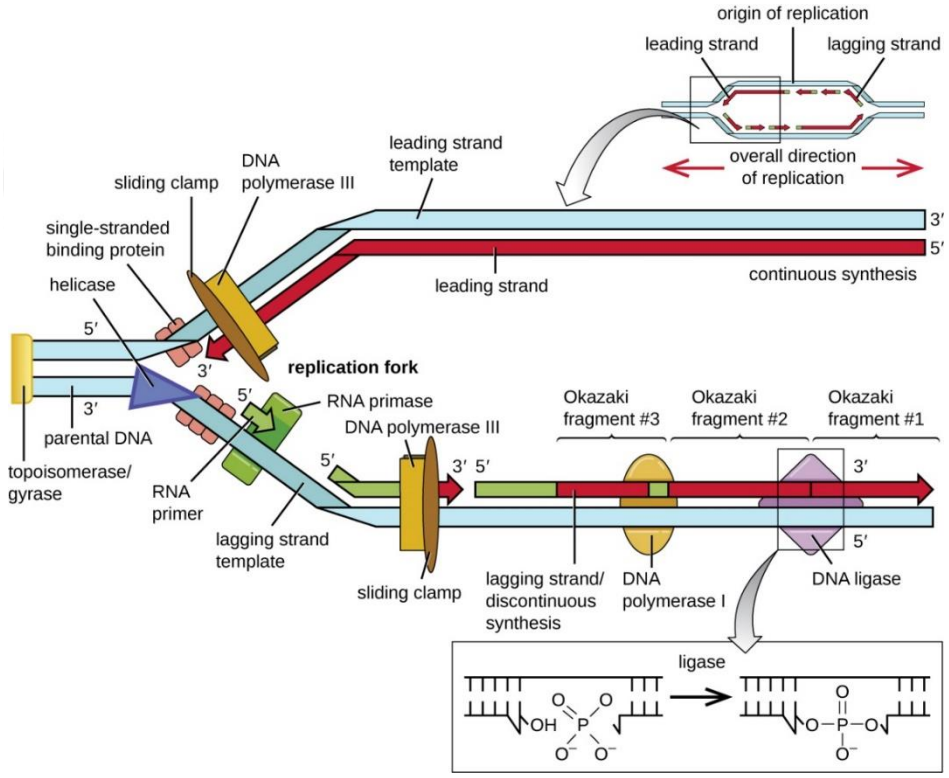

Figure 6. At the origin of replication, topoisomerase II relaxes the supercoiled chromosome. Two replication forks are formed by the opening of the double-stranded DNA at the origin, and helicase separates the DNA strands, which are coated by single-stranded binding proteins to keep the strands separated. DNA replication occurs in both directions. An RNA primer complementary to the parental strand is synthesized by RNA primase and is elongated by DNA polymerase III through the addition of nucleotides to the 3'-OH end. On the leading strand, DNA is synthesized continuously, whereas on the lagging strand, DNA is synthesized in short stretches called Okazaki fragments. RNA primers within the lagging strand are removed by the exonuclease activity of DNA polymerase I, and the Okazaki fragments are joined by DNA ligase.

From https://openstax.org/books/microbiology/pages/11-2-dna-replication

Once the complete chromosome has been replicated, **termination of DNA replication** must occur. Although much is known about initiation of replication, less is known about the termination process.

Following replication, the resulting complete circular genomes of prokaryotes are concatenated, meaning that the circular DNA chromosomes are interlocked and must be separated from each other. This is accomplished through the activity of bacterial topoisomerase IV, which introduces double-stranded breaks into DNA molecules, allowing them to separate from each other; the enzyme then reseals the circular chromosomes.

The resolution of concatemers is an issue unique to prokaryotic DNA replication because of their circular chromosomes. Because both bacterial DNA gyrase and topoisomerase IV are distinct from their eukaryotic counterparts, these enzymes serve as targets for a class of antimicrobial drugs called quinolones.

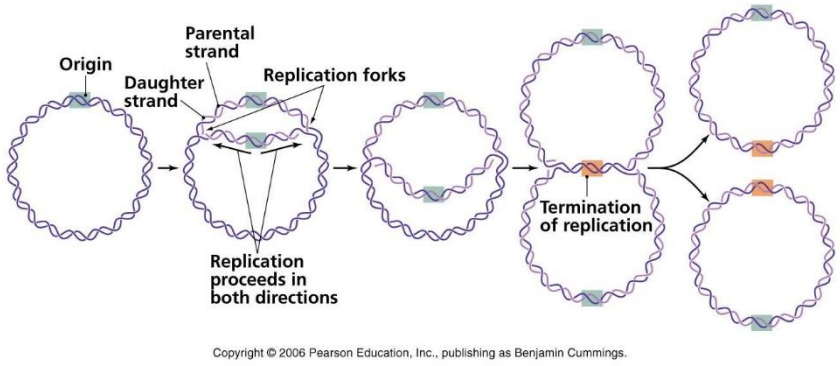

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Figure 7. The two new daughter DNA circles will be separated by DNA topoisomerase II during the termination of replication.

Fidelity of base incorporation is dependent on Watson-Crick base-pairing (A-T, G-C), plus purine-pyrimidine matching. Incorrectly incorporated bases are removed by proofreading of DNA polymerase via its 3' to 5' exonuclease activity. This activity is found in most polymerases that are used to replicate DNA in cells. A different polymerase removes RNA primers that were used to initiate DNA replication using 5' -> 3' exonuclease activity.

Eukaryotic Replication

Our understanding of replication in eukaryotes is not as extensive as that in prokaryotes, owing to their higher level of complexity of cellular organization. Even though many of the principles are the same, eukaryotic replication is more complicated in three basic ways: there are multiple origins of replication, the timing must be controlled to that of cell divisions, and more proteins and enzymes are involved. The table below summarizes the differences between prokaryotic and eukaryotic replication.

Table 10.5

Differences in DNA Replication in Prokyryotes and Eukaryotes

Prokaryotes	Eukaryotes
Five polymerases (I, II, III, IV, V)	Five polymerases (α, β, γ, δ, ε)
Functions of polymerase:	Functions of polymerases:
I is involved in synthesis, proofreading, repair, and removal of RNA primers	α: a polymerizing enzyme
II is also a repair enzyme	β: is a repair enzyme
III is main polymerizing enzyme	γ: mitochondrial DNA synthesis
IV, V are repair enzymes under unusual conditions	δ: main polymerizing enzyme ε: function unknown
Polymerases are also exonucleases	Not all polymerases are exonucleases
One origin of replication	Several origins of replication
Okazaki fragments 1000-2000 residues long	Okazaki fragments 150-200 residues long
No proteins complexed to DNA	Histones complexed to DNA

Eukaryotic replication utilizes a different set of DNA polymerase enzymes. Several of the DNA polymerases isolated from animals lack exonuclease, or proofreading, activity (the α and β enzymes). This function appears to be carried out by separate exonucleolytic enzymes exist in animal cells.

Table 10.4
The Biochemical Properties of Eukaryotic DNA Polymerases

	α	δ	ε	β	γ
Mass (kDa)					
Native	>250	170	256	36-38	160-300
Catalytic core	165-180	125	215	36-38	125
Other subunits	70, 50, 60	48	55	None	35, 47
Location	Nucleus	Nucleus	Nucleus	Nucleus	Mitochondria
Associated functions					
3' → 5' exonuclease	No	Yes	Yes	No	Yes
Primase	Yes	No	No	No	No
Properties					
Processivity	Low	High	High	Low	High
Fidelity	High	High	High	Low	High
Replication	Yes	Yes	Yes	No	Yes
Repair	No	?	Yes	Yes	No

Source: Adapted from Kornberg, A., and Baker, T. A., 1992. *DNA Replication*, 2nd ed. New York: W. H. Freeman and Co.

DNA replication in eukaryotes takes place during the S phase. Even though eukaryotic chromosomes are much longer than prokaryotic chromosomes, the replication process can finish quite rapidly due to the presence of multiple origins of replication. Multiple bidirectional replication forks simultaneously expand and will eventually merge. The zones where replication is proceeding are called **replicons,** and the size of these varies with the species. In higher mammals, replicons may span 500 to 50,000 base pairs.

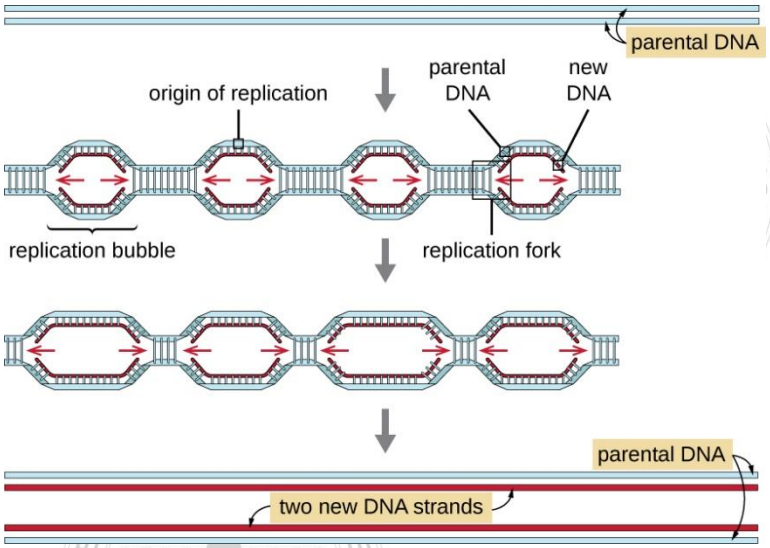


Figure 8. Eukaryotic chromosomes are typically linear, and each contains multiple origins of replication. From https://openstax.org/books/microbiology/pages/11-2-dna-replication

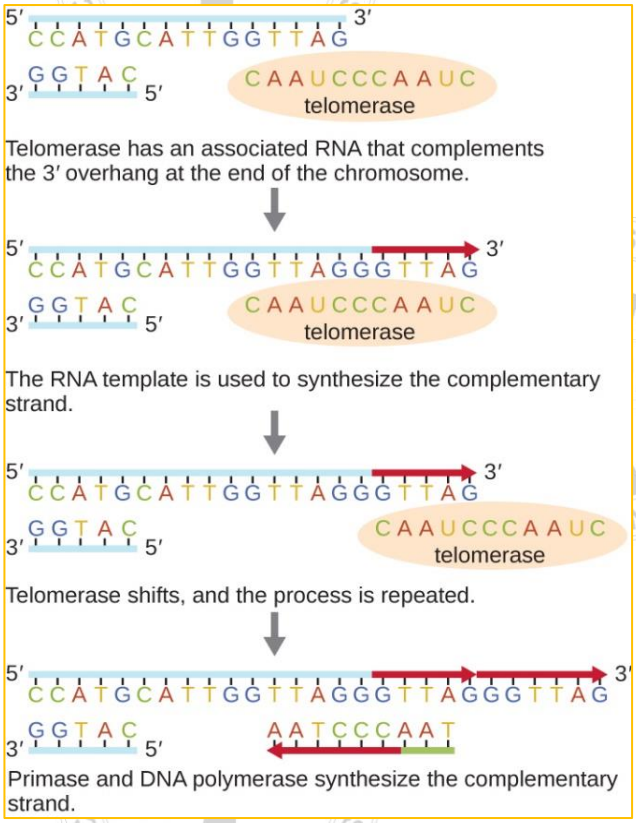
In addition, in eukaryotes, the DNA template is complexed with histone proteins into nucleosomal structures. Histone biosynthesis occurs at the same time and at the same rate

as DNA biosynthesis. In eukaryotic replication, histones are associated with DNA as it is formed.

The end – replication problem in eukaryotes

As in prokaryotes, the eukaryotic DNA polymerase can add nucleotides only in the 5' to 3' direction, and requires primers from which to initiate DNA replication. In the leading strand, synthesis continues until it reaches either the end of the chromosome or another replication fork progressing in the opposite direction. Synthesis of the lagging strand also requires a short primer which will later be removed and replaced by DNA. However, at the extreme end of a chromosome, there is no way to synthesize this region when the last primer is removed. Therefore, the lagging strand is always shorter than its template by at least the length of the primer. This is the so-called "end-replication problem". After every round of replication, a eukaryotic chromosome loses a bit of the DNA at its ends.

The end – replication problem is solved by the presence of telomeres, which are non – coding DNA segments composed of the tandem repeat sequence TTAGGG. Because these sequences do not carry information vital to the cell's survival, their loss after each round of replication does not usually harm the cell. However, once telomeres shrink to certain level, the cell can no longer divide. Healthy human cells can divide only a finite number of times before they reach senescence, where the cell is unable to divide. Telomeres are the basis for the number of times a cell is able to divide before reaching senescence.



Chromosome shortening does not occur in germ cells (cells producing eggs and sperm) because of the activity of **telomerase** enzyme, which adds new telomere sequences onto the ends of newly replicated DNA. Telomerase contains an RNA component which is complementary to the telomere repeat sequence. Hence, the internal RNA can serve as the template for synthesizing DNA. Telomerase activity can extend telomeres by many repeats.

For her discovery of telomerase and its action, Elizabeth Blackburn received the Nobel Prize for Medicine or Physiology in 2009.

Figure 9. In eukaryotes, the ends of the linear chromosomes are maintained by the action of the telomerase enzyme. from https://openstax.org/books/microbiology/pages/11-2-dna-replication

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https://www.nature.com/scitable/topicpage/major-molecular-events-of-dna-replication-413/

http://oregonstate.edu/instruction/bi314/fall11/dnarep.html

https://www.web-books.com/MoBio/Free/Ch7D.htm

ADDITIONAL VIDEO RESOURCES

- Structural Basis of DNA Replication HD Animation https://www.youtube.com/watch?v=FUt6U58EA8w&list=PLYCGVJq0DVwKrmoSIvhzAOh0SpNUgfqFf&index=19
- DNA replication - 3D https://www.youtube.com/watch?v=TNKWgcFPHqw
- How Nucleotides are added in DNA Replication HD Animation https://www.youtube.com/watch?v=9QtQrVyiEK0&list=PLYCGVJq0DVwKrmoSIvhzAOh0SpNUgfqFf&index=51
- DNA Replication Fork 2 HD Animation https://www.youtube.com/watch?v=2im5HEw2de0&list=PLYCGVJq0DVwKrmoSIvhzAOh0SpNUgfqFf&index=65
- Bidirectional Replication of DNA HD Animation https://www.youtube.com/watch?v=2JazZ0BAm-w&list=PLYCGVJq0DVwKrmoSIvhzAOh0SpNUgfqFf&index=83
- Action of DNA Gyrase HD Animation https://www.youtube.com/watch?v=9j-f2kovgnU&list=PLYCGVJq0DVwKrmoSIvhzAOh0SpNUgfqFf&index=87
- Proofreading of DNA polymerase HD Animation https://www.youtube.com/watch?v=BIO0VW2_GTQ&list=PLYCGVJq0DVwKrmoSIvhzAOh0SpNUgfqFf&index=37