

Prokaryotic DNA Replication: Enzymes and Their Function

Enzyme/protein	Specific Function
8. Topoisomerase	Helps relieve the stress on DNA when unwinding by causing breaks and then resealing the DNA
9. Single-strand binding proteins (SSB)	Binds to single-stranded DNA to avoid DNA rewinding back.

DNA Replication in Eukaryotes

Eukaryotic genomes are much more complex and larger in size than prokaryotic genomes. The human genome has three 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 the eukaryotic chromosome; humans can have up to 100,000 origins of replication. 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*.

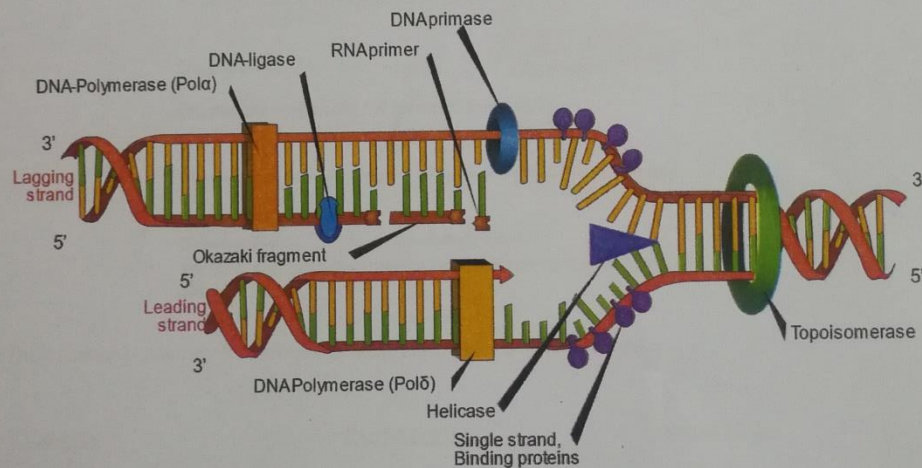
The number of DNA polymerases in eukaryotes is much more than prokaryotes: 14 are known, of which five are known to have major roles during replication and have been well studied. They are known as pol α , pol β , pol γ , pol δ , and pol ϵ .

The essential steps of replication are the same as in prokaryotes. Before replication can start, the DNA has to be made available as template. Eukaryotic DNA is bound to basic proteins known as histones to form structures called nucleosomes. 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. Other proteins are then recruited to start the replication process. 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. 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. RNase H removes the RNA primer, which is then replaced with DNA nucleotides. The Okazaki fragments in the lagging strand are joined together after the

replacement of the RNA primers with DNA. The gaps that remain are sealed by DNA ligase, which forms the phosphodiester bond.

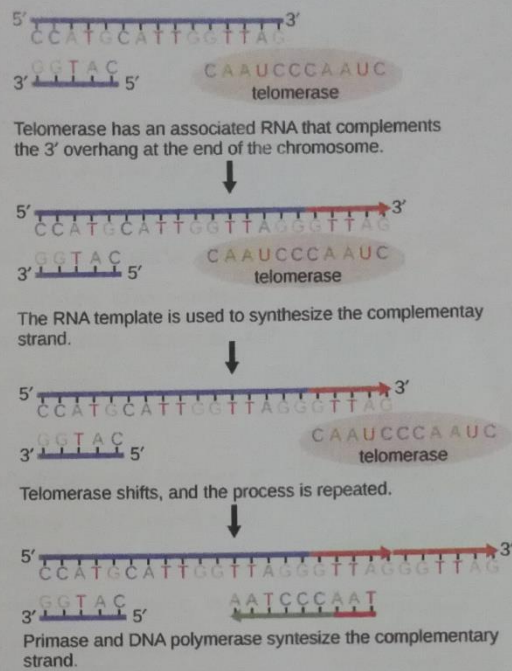
Telomere replication

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 place for a primer to be made for the DNA fragment to be copied at the end of the chromosome. These ends thus remain unpaired, and over time these ends may get progressively shorter as cells continue to divide.



The ends of the linear chromosomes are known as **telomeres**, which have repetitive sequences that code for no particular gene. In a way, these telomeres protect the genes from getting deleted as cells continue to divide.

In humans, a six base pair sequence, TTAGGG, is repeated 100 to 1000 times. The discovery of the enzyme telomerase helped in the understanding of how 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 complementary bases 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.



Difference between Prokaryotic and Eukaryotic Replication

<u>Property</u>	<u>Prokaryotes</u>	<u>Eukaryotes</u>
1. Origin of replication	Single	Multiple
2. Rate of replication	1000 nucleotides/s	50 to 100 nucleotides/s
3. DNA polymerase types	5	14
4. Telomerase	Not present	Present
5. RNA primer removal	DNA pol I	RNase H
6. Strand elongation	DNA pol III	Pol δ , pol ϵ
7. Sliding clamp	Sliding clamp	PCNA