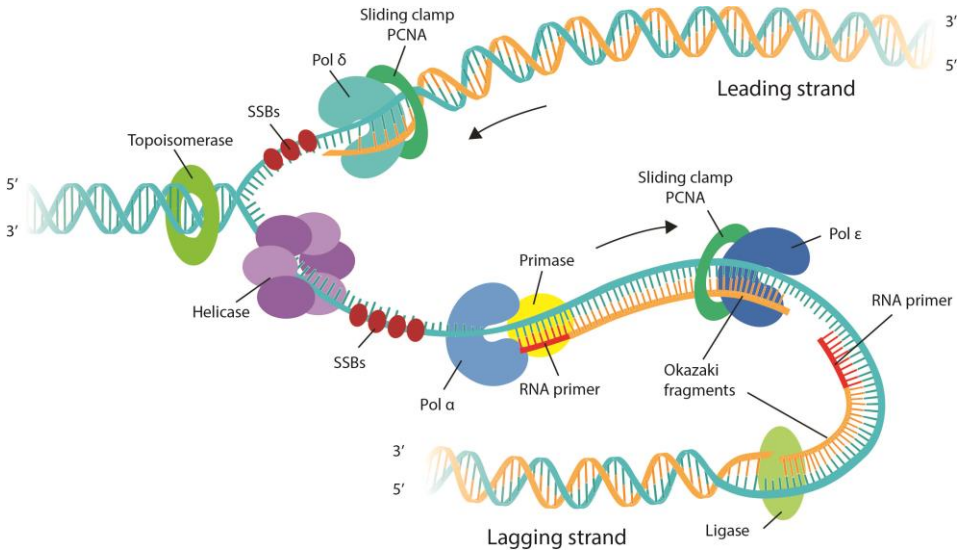
DNA replication in Eukaryotes cells:

DNA replication in eukaryotes occur only in S-phase of cell cycle. However pre-initiation occur in G1 pahse. Due to sheer size of chromosome in eukaryotes, chromosome chromosome contains multiple origin of replication. ARS (autonomously replicating sequence) in case of yeast is origin for replication.

Steps in DNA replication:

1. Initiation:

- The first steps is the formation of pre-initiation replication complex (pre-RC). is binding of ORC (origin recognition complex).
- The replication begins with binding of ORC to the origin. ORC is a hexamer of related protein and remains bounded even after DNA replication occurs. Furthermore ORC is analogue of prokaryotic dnaA protein.
- After binding of ORC to origin, cdc6/cdc18 and cdtl coordinate the loading of MEM (mini chromosome maintainance) to origin.
- MEM complex is thought to be major eukaryotic helicase.
- After binding of MEM complex to pre-RC, cdtl get displaced. Then DdK phosphorylates MEM, which activates its helicase activity. Again DdK and CdK recruit another protein called cdc45 which then recruit all the DNA replicating protein such that the origin get fired and replication begins.



2. Elongation:

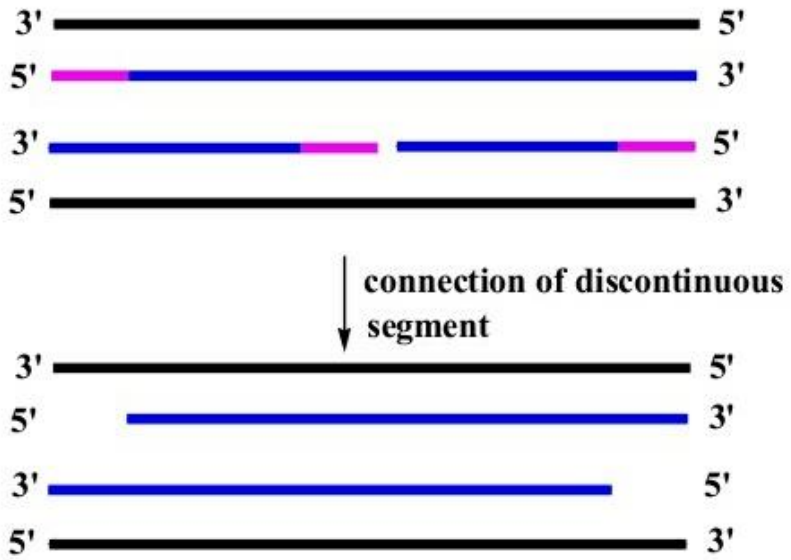
- DNA polymerase δ synthesizes and adds dNTPs at 3’ end of RNA primer.
- The leading and lagging strands are synthesized in the similar fashion as in prokaryotic DNA replication.

3. Termination:

- At the end of DNA replication the RNA primer are replaced by DNA by 5’-3’exonuclease and polymerase activity of DNA polymerase ε.
- Exonuclease activity of DNA polymerase removes the RNA primer and polymerase activity adds dNTPs at 3’-OH end preceding the primer.
- In case of bacteria, with circular genome, the replacement of RNA primer with DNA is not a problem because there is always a preceding 3’-OH in a circular DNA.
- But in eukaryotic organism with linear DNA, there is a problem. When RNA primer at 5’ end of daughter strand is removed, there is not a preceding 3’-OH such that the DNA polymerase can use it to

replace by DNA. So, at 5' end of each daughter strand there is a gap (missing DNA). This missing DNA cause loss of information contain in that region. This gap must be filled before next round of replication.

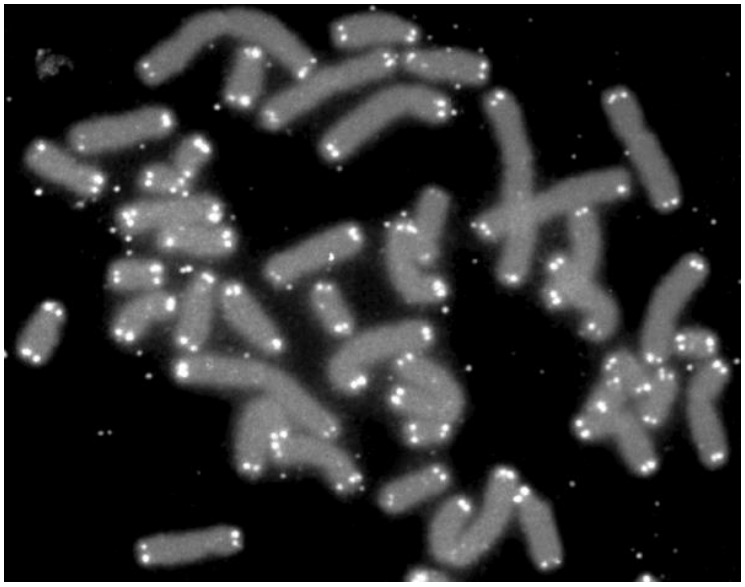
- For solving this end replication problem ; studies have found that linear end of DNA called telomere has G:C rich repeats. These sequence is known as telomere sequence. These repeats of telomere sequence is different among different organisms. Telomere in human cell consists of repeats of TTAGGG/AATCCC. Each species has its own species specific telomere repeats. These telomere sequence donot codes anything but it is essential to fill the gap in daughter strand and maintain the integrity of DNA.



Telomeres and telomerase

Telomeres as protective "caps" on the tips of eukaryotic chromosomes. How telomerase extends telomeres.

If you could zoom in and look at the DNA on the tip of one of your chromosomes, what would you see? You might expect to find genes, or perhaps some DNA sequences involved in gene regulation. Instead, what you'd actually find is a single sequence –TTAGGG – repeated over and over again, hundreds or even thousands of times.



Repetitive regions at the very ends of chromosomes are called **telomeres**, and they're found in a wide range of eukaryotic species, from human beings to unicellular protists. Telomeres act as caps that protect the internal regions of the chromosomes, and they're worn down a small amount in each round of DNA replication.

The end-replication problem

Unlike bacterial chromosomes, the chromosomes of eukaryotes are linear (rod-shaped), meaning that they have ends. These ends pose a problem for DNA replication. The DNA at the very end of the chromosome cannot be

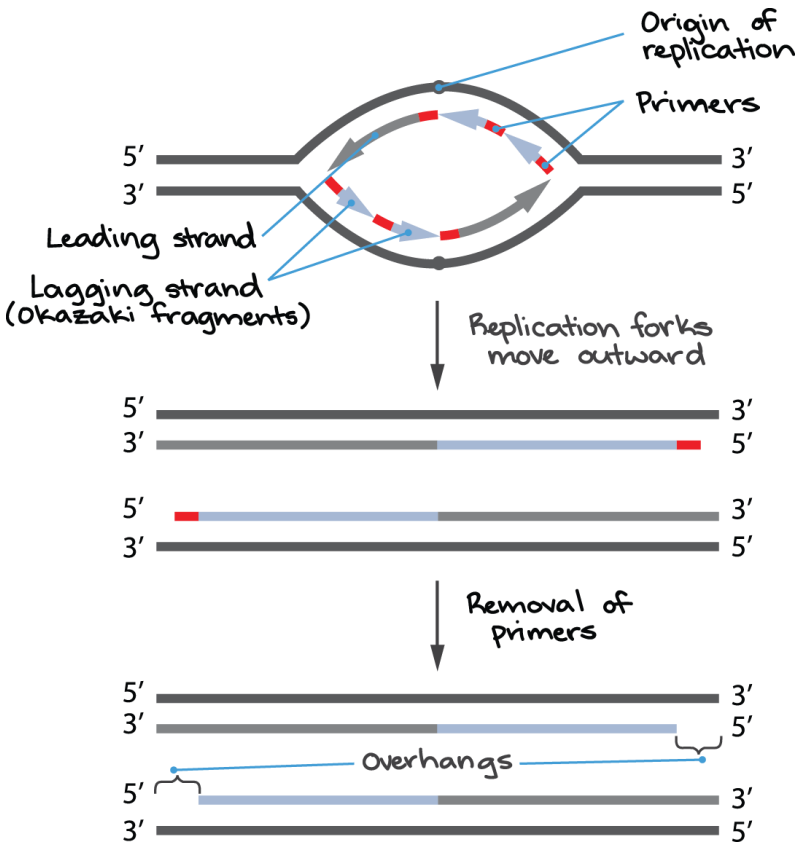
fully copied in each round of replication, resulting in a slow, gradual shortening of the chromosome.

Why is this the case? When DNA is being copied, one of the two new strands of DNA at a replication fork is made continuously and is called the **leading strand**. The other strand is produced in many small pieces called Okazaki fragments, each of which begins with its own RNA primer, and is known as the **lagging strand**.

In most cases, the primers of the Okazaki fragments can be easily replaced with DNA and the fragments connected to form an unbroken strand. When the replication fork reaches the end of the chromosome, however, there is (in many species, including humans) a short stretch of DNA that does not get covered by an Okazaki fragment—essentially, there's no way to get the fragment started because the primer would fall beyond the chromosome end. Also, the primer of the last Okazaki fragment that *does* get made can't be replaced with DNA like other primers.

Part of the DNA at the end of a eukaryotic chromosome goes uncopied in each round of replication, leaving a single-stranded overhang. Over multiple rounds of cell division, the chromosome will get shorter and shorter as this process repeats.

In human cells, the last RNA primer of the lagging strand may be positioned as much as 70 to 100 nucleotides away from the chromosome . Thus, the single-stranded overhangs produced by incomplete end replication in humans are fairly long, and the chromosome shortens significantly with each round of cell division.

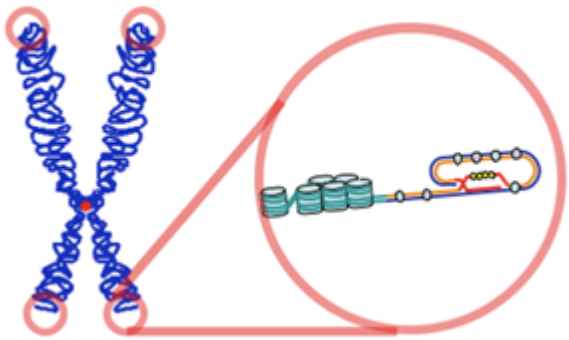


Telomeres

To prevent the loss of genes as chromosome ends wear down, the tips of eukaryotic chromosomes have specialized DNA "caps" called **telomeres**. Telomeres consist of hundreds or thousands of repeats of the same short DNA sequence, which varies between organisms but is 5'-TTAGGG-3' in humans and other mammals.

Telomeres need to be protected from a cell's DNA repair systems because they have single-stranded overhangs, which "look like" damaged DNA. The overhang at the lagging strand end of the chromosome is due to incomplete end replication (see figure above). The overhang at the leading strand end of the chromosome is actually generated by enzymes that cut away part of the DNA. In some species (including humans), the single-stranded overhangs bind to complementary repeats in the nearby double-

stranded DNA, causing the telomere ends to form protective loops. Proteins associated with the telomere ends also help protect them and prevent them from triggering DNA repair pathways.



The repeats that make up a telomere are eaten away slowly over many division cycles, providing a buffer that protects the internal chromosome regions bearing the genes (at least, for some period of time). Telomere shortening has been connected to the aging of cells, and the progressive loss of telomeres may explain why cells can only divide a certain number of times.

Telomerase

Some cells have the ability to reverse telomere shortening by expressing **telomerase**, an enzyme that extends the telomeres of chromosomes. Telomerase is an RNA-dependent DNA polymerase, meaning an enzyme that can make DNA using RNA as a template.

How does telomerase work? The enzyme binds to a special RNA molecule that contains a sequence complementary to the telomeric repeat. It extends (adds nucleotides to) the overhanging strand of the telomere DNA using this complementary RNA as a template. When the overhang is long enough, a matching strand can be made by the normal DNA replication

machinery (that is, using an RNA primer and DNA polymerase), producing double-stranded DNA.

The primer may not be positioned right at the chromosome end and cannot be replaced with DNA, so an overhang will still be present. However, the overall length of the telomere will be greater.

Telomerase is not usually active in most somatic cells (cells of the body), but it's active in germ cells (the cells that make sperm and eggs) and some adult stem cells. These are cell types that need to undergo many divisions, or, in the case of germ cells, give rise to a new organism with its telomeric "clock" reset. Interestingly, many cancer cells have shortened telomeres, and telomerase is active in these cells. If telomerase could be inhibited by drugs as part of cancer therapy, their excess division (and thus, the growth of the cancerous tumor) could potentially be stopped.