2.5 DNA REPLICATION IN EUKARYOTES ⑦

DNA replication in eukaryotes occus in nucleous. Eukaryotic DNA is bound to proteins known as histones. Eukaryotic DNA is this linear and complex DNA and it requires multiple origin of replication. It occurs in S-phase of cell cycle. The huge genome of eukaryotes is replicated in small portions called as replicons. The Ori site has specific conserved sequence.

These are three steps which are involved in the replication of eukaryotic DNA:

(*i*) Initiation

(*ii*) Elongation

(*iii*) Termination

(*i*) **Initiation:** The initial step of DNA replication is to find out a specific site for origin of replication. As the eukaryotic DNA is larger in size than prokaryotic so it consists of multiple sites of origin of replication. These Ori sites are AT-rich sequences. In yeast, these AT-rich sequences ar called as (ARS) autonomously replicating sequences. There are various proteins which are involved during initiation.

TABLE 2.3 *Proteins involved during initiation of eukaryotic DNA replication.*

S.No.	Protein	Name	Function
1.	ORC	Origin replication complex	It binds to ori site and AT pase actiite & recruite all other protein for initiation.
2.	CDC 6	Cell division cycle 6	It requires for helicase loading
3.	CDT 1	CDC 10 dependant transcript	It is used for MCM loading and interacts with it.
4.	MCM 2-7	Mini chromosome maintenance	It is a hexamer of MCM protein & has helicase activity
5.	CDK/DDK	Cycle dependant primase and dbf4 dependent kinase	They both regulate the initation process by phosphorylation
6.	CDC-45	Cell division cycle 45	It interacts with MCM 7 and polymerase
7.	RPA	Replication protein A	It prevents reannealing of DNA strands as SSBs
8.	RFC	Replication factor C	DNA clamp loader. It loads PCNA to the DNA strands
9.	PCNA	DNA sliding clamp holds to DNA	Proliferating cell nuclear antigen
10.	DNA pol α		Initiates DNA synthesis on primers
11.	DNA pol δ		Synthesis of leading and lagging strand
12.	Primase		Adds primers to lagging strand

The initiation of eukaryotic DNA requires 6 subunit complex where origin recognition complex (ORC) serves as the attachment site for several other proteins. This is analogous to Dna A protein in prokaryotes. There are 2 proteins bound to this ORC site, first is replication activator protein (RAP)and second protein is (RLF) replication licensing factor. Unless these proteins binds to ORC site DNA replication could not begin. This assembly is called as pre-replication complex (pre-RC). All events occurs in G1 phase of cell-cycle.

The double-stranded DNA molecule needs to be separated for the synthesis of new strand. In prokaryotes, the DnaB protein acts as a helicase, whereas in eukaryotes the MCM (Mini-Chromosome Maintenance) protein complex functions as helicase. MCM is a hexameric protein that unwinds the DNA at the replication fork. Once the hydrogen bonds are broken, single-stranded binding proteins (SSBs) bind to prevent the reannealing of DNA strands. In eukaryotes, SSBs are called replication protein A (RPA) and serve as single-stranded binding proteins. The primase enzyme adds primers to both strands. A short stretch of RNA primer is added by primase to initiate synthesis on the leading strand. On the lagging strand, multiple primers are required because synthesis occurs discontinuously, forming Okazaki fragments. After the primers are added, DNA polymerase incorporates nucleotides on both strands.

(*ii*) **Elongation:** Once polymerase α attaches a few nucleotides at both the ends, it is further elongated by certain enzymes in the elongation process proliferating cell nuclear antigen (PCNA) a DNA clamp which loads the DNA polymerase to both the strands. So on one strand DNA polymerase ε synthesize continuously called as leading strand and on the other strand DNA polymerase δ synthesize in discontinuous manner forming okazaki fragments on lagging strand. DNA produces supercoiling which is removed by topoisomerase. After the elongation is complete there is as need to remove primers. In case of prokaryotes DNA pol I removes the primers whereas in eukaryotes primers are removed by FEN-1 endonuclease. Once the primers are removed a gap is produced which is filled by DNA pol δ and sealed by DNA ligase.

(iii) **Termination:** Termination of eukaryotes is different and complex than prokaryotes. Prokaryotic DNA is double-stranded circular and termination occurs at 'Ter' sequences whereas in eukaryotes have complex linear chromosomes. The DNA at the end of the eukaryotic chromosome cannot be fully copied in each round of replication. When the primers are removed it leaves the ends unpredicted, in each round of replication, leaving a single–stranded overhang. Over multiple rounds of cell-division, the chromosome of eukaryotes would get shorter and shorter.

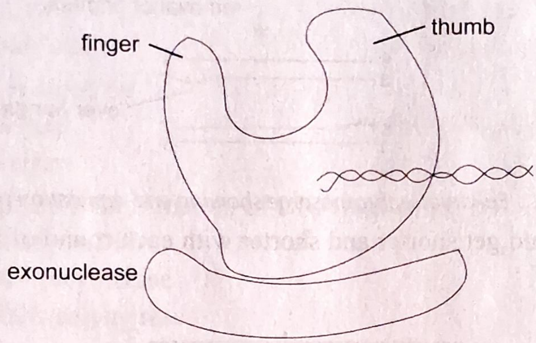

Fig. 2.11. *Structure of DNA polymerase III.*

To prevent the loss of genes at the tips of eukaryotic chromosome have specialized DNA 'caps' called 'telomeres'. The telomeres are composed of repeats of short, GC rich sequences.the G-rich strand of a telomeres is added at the very 3′ ends of DNA strands, not by semi conservative replicate, but by an enzyme called telomerase, Telomeres consists of hundreds or thousands of repeats of the Same short DNA sequences. The telomeres sequence in humans and other mammals is 5′- TTAGGG-3′. The enzyme telomerase have the ability to reverse telomeres shortening. It is a RNA-dependent DNA polymerase that can make DNA using RNA as a template. The enzyme binds to that sequence which is complementary to the telomeric repeat. Telomerase adds nucleotides to the overhanging strand of the telomer DNA and is called as reverse transcriptase. Telomerase is usualy active in germ cells and least found in somatic cells. It is a ribonucleo protein with essential RNA and protein sub units. Telomerase is important for maintaining chromosome integrity.

S. No.	***Organism***	***Telomeric repeat***
1.	Human, mammals, birds.....	TTAGGG
2.	Trypanosomes	TTAGGG
3.	*Holotrichous ciliates*	GGGGTT
4.	Yeast	GT,GGT,GGGT
5.	Plants	TTTAGGG

Telomeres comes from two Greek word which has Telo means 'end' and meros means 'part'. So it is the end part of the chromosome or DNA.

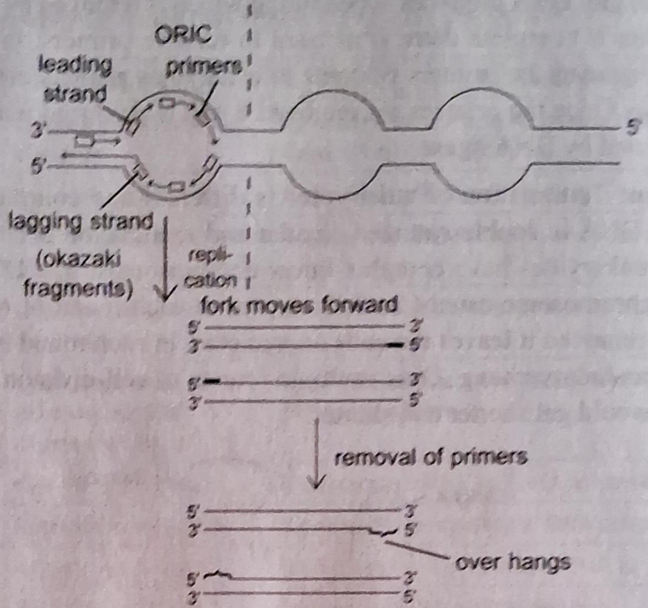

Fig. 2.12. Eukaryotic chromosome showing end-replication problem.

Chromome ends would get shorter and shorter with each round of replication is called the 'end replication problem'

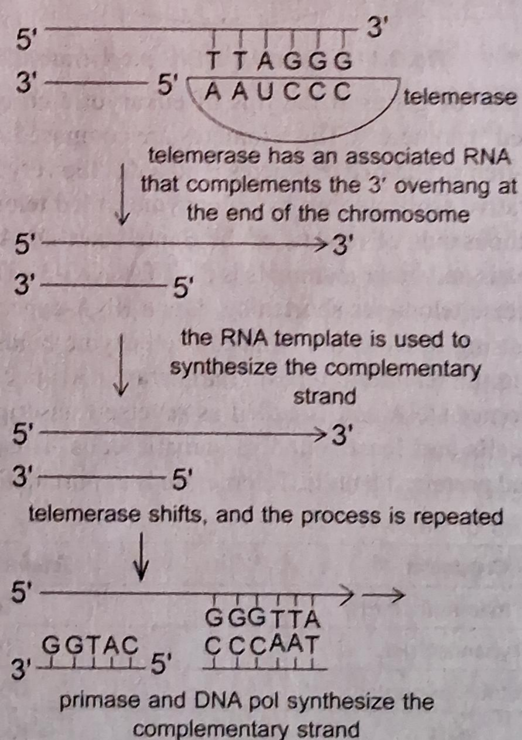

Fig. 2.13. The end replication problem is solved by the action off the telomerase enzyme.

The end replication problem is solved by telomerase because the number of times a normal human Cell divides a part of telomeres are lost every time. The loss of telomeres is about 300 to 1000 ntds with each round of cell-division. It divides until so much of the chromosomes ends have lost such that cells can no longer divide and is called senescence where a cell can no longer divide and this is regarded as **Hay flick limit**. Hay flick limit is the number of times a normal human cell divide until no longer DNA is remaining. It is about 50 to 70 cell divisions and so once the cell reaches this Hay flick limit or reach senescence the cells age. As the cells age, the organism will also age and in eukaryotes this is the way half cell leads to the process of aging.