VIVEKANANDA COLLEGE
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NAAC ACCREDITED 'A' GRADE

Topic:Replication of genome:Prokaryotic Termination and Eukaryotic Replication(Briefly)

Course Title: Gene Organization, Expression and Regulation

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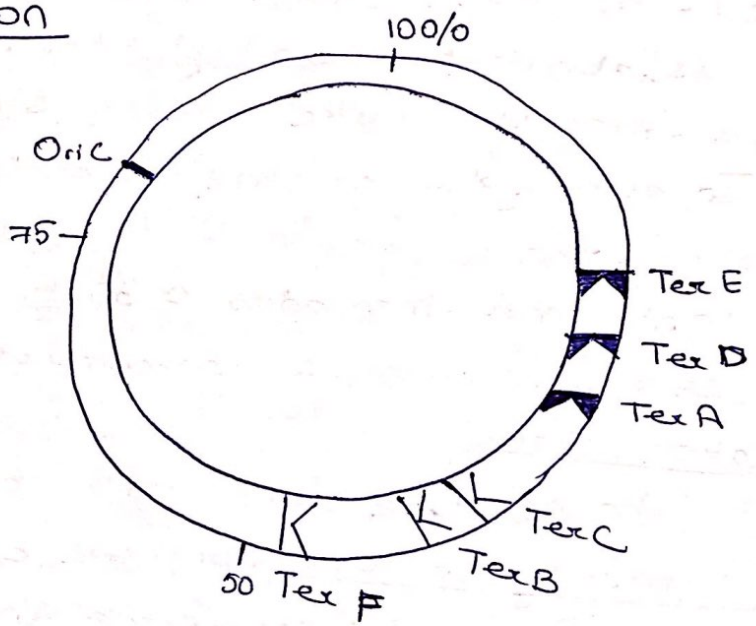
Figure No. .....

The adjacent okazaki fragments must attach to form a complete strand but each fragment has a RNA primer where ligation takes place, here DNA Pol III releases the lagging strand and DNA Pol I which has 5'→3' exonuclease activity continues synthesis. It removes the primer and the fragments are joined by DNA ligase to complete the lagging strand synthesis.

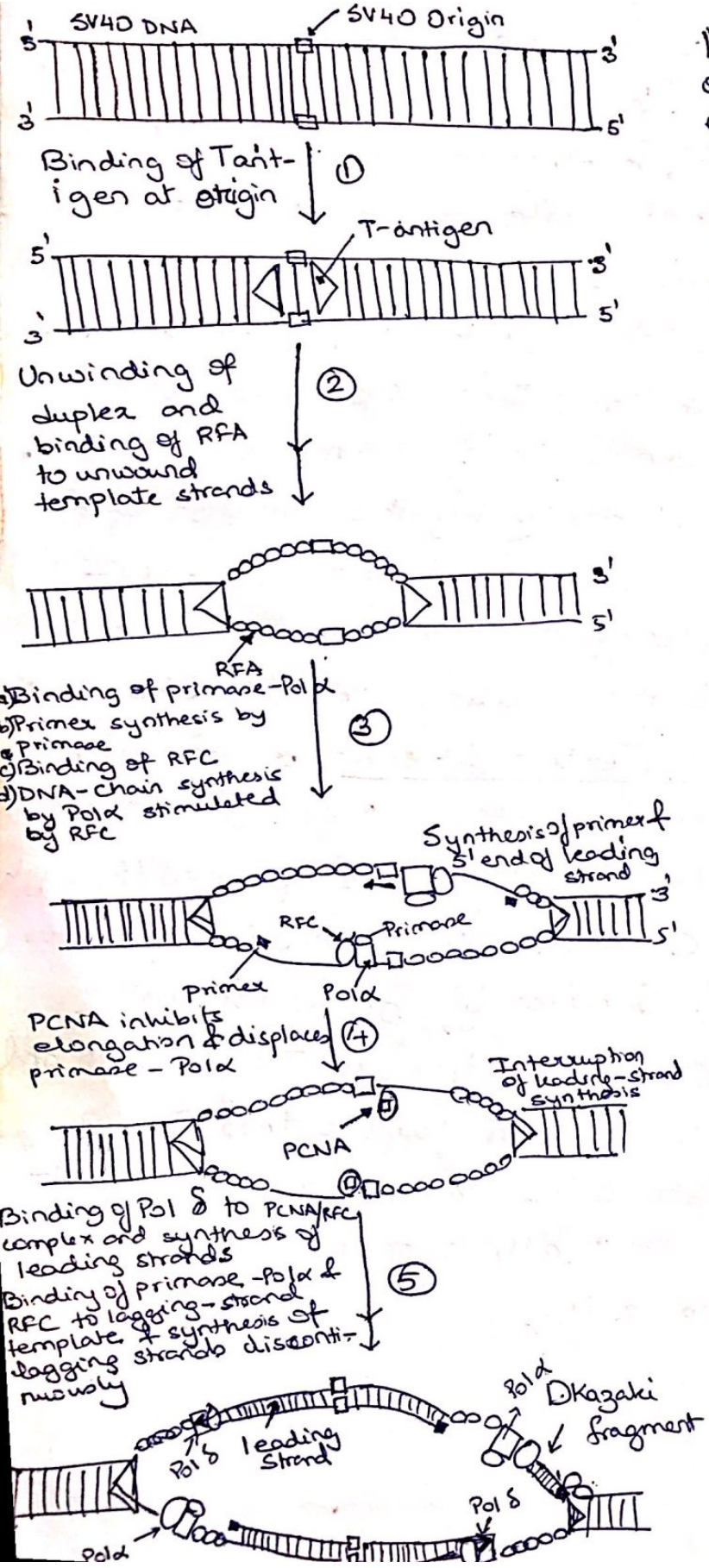
Termination



Bacterial genomes are replicated bidirectionally from a single point and 2 replication forks meet at a point diametrically opposite to Ori C, if one fork is delayed the other fork is not allowed to overshoot the ½ way point and continue replication. This is due to the presence of termination sequence, which acts as a recognition site for the sequence, (which acts) specific DNA binding protein called Tus protein, (a 309 residue monomer), a product of Tus gene. Tus protein binds at the ter site and prevent strand

displacement by Dna B helicase thereby preventing replication fork motion. When a tus protein binds with a DNA-binding protein, replication is inhibited at the other proteins binding site, which suggests that tus protein does not act as a sliding clamp but interacts with the Dna B helicase to prevent its helicase action. Curiously, however, this termination system is not essential for termination, other replication terminus is absent, replication is inhibited simply by the collision of 2 replication fork.

The E. coli replication terminus is large (350 flanked by 6 identical non-palindromic ~23bp termination sites, Ter E, Ter D and Ter A on one side and Ter F, Ter B, Ter C on the other side. A replication fork travelling in counter-clockwise passes through Ter F, Ter B, Ter C stops on encountering either Ter A, Ter D or Ter E. Similarly, on clockwise travelling replication forks transits Ter A, Ter D and Ter E but halts at Ter C or failing that Ter B or Ter F. Termination sites are polar which acts as a 1-way valves that allow replication forks to enter but not leave it.



Model of in vitro replication of SV40 DNA by eukaryotic enzymes

In contrast to replication in E. coli, 2 diff DNA polymerases catalyse elongation of the leading and lagging strands. Polymerase α (Pol α), which is tightly associated with a primase, forms the 5' ends of the leading strands and then is displaced from the template. Association of Pol δ with PCNA increases the enzyme's procesivity, so that it can synthesize the remainder of the leading strands. Lagging-strand synthesis downstream from the leading strand primers is thought to be carried out by the combined action of primase and Pol α. RFC stimulates the activity of Pol α.

RFA = Replication factor A

RFC = u u C

PCNA = proliferating cell nuclear antigen.

There are 5 mammalian DNA polymerases

DNA Polymerase	α (I)	δ (III)	ε (II)	β	γ
Location	Nuclear	Nucleus	Nucleus	Nucleus	Mitochondrial
Function	Lagging strand synthesis & priming	Leading strand synthesis	Repair	Repair	Replication
Mass (daltons) & Subunits	300,000 Catalytic core 2 primase 1 unknown	170-230,000 Catalytic core 1 unknown requires PCNA	250,000 Catalytic core 1 unknown	40,000 Catalytic core	180-300,000 Catalytic core 2 unknown
3'→5' exonuclease	No	Yes	Yes	No	Yes
Dideoxythymidine	No effect	No effect	Weak	Inhibitory	Inhibitory
Aphidicolin	Inhibitory	Inhibitory	Inhibitory	No effect	No effect
Polymerization 5'→3'	+	+	+	+	+
Exonuclease activity 3'→5'	-	+	+	-	+
Synthesis from					
1) RNA primer	+	+	?	-	-

The End Problem of Linear DNA Replication

Linear chromosomes have an end problem. After DNA replication, each newly synthesized DNA strand is shorter at its 5′ end than at the parental DNA strand's 5′ end. This produces a 3′ overhang at one end (and one end only) of each daughter DNA strand, such that the two daughter DNAs have their 3′ overhangs at opposite ends.

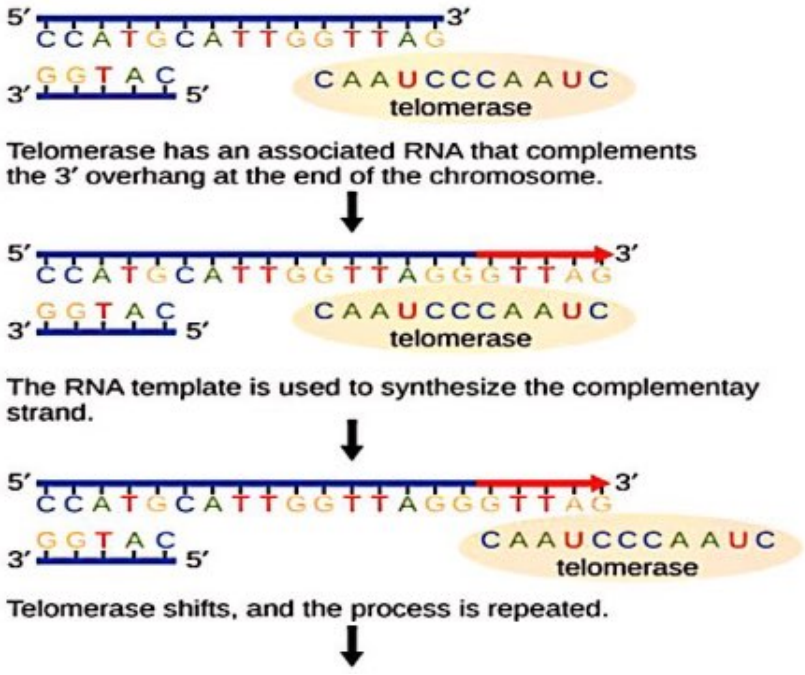
Telomere Replication

The ends of the linear chromosomes are known as telomeres: repetitive sequences that code for no particular gene. These telomeres protect the important genes from being deleted as cells divide and as DNA strands shorten during replication.

In humans, a six base pair sequence, TTAGGG, is repeated 100 to 1000 times. After each round of DNA replication, some telomeric sequences are lost at the 5′ end of the newly synthesized strand on each daughter DNA, but because these are noncoding sequences, their loss does not adversely affect the cell. However, even these sequences are not unlimited. After sufficient rounds of replication, all the telomeric repeats are lost, and the DNA risks losing coding sequences with subsequent rounds.

The discovery of the enzyme telomerase helped in the understanding of how chromosome ends are maintained. The telomerase enzyme attaches to the end of a chromosome and contains a catalytic part and a built-in RNA template. Telomerase adds complementary RNA bases to the 3′ end of the DNA strand. Once the 3′ end of the lagging strand template is sufficiently elongated, DNA polymerase adds the complementary nucleotides to the ends of the chromosomes; thus, the ends of the chromosomes are replicated.

Telomerase is important for maintaining chromosome integrity: The ends of linear chromosomes



Telomerase and Aging

Telomerase is typically active in germ cells and adult stem cells, but is not active in adult somatic cells. As a result, telomerase does not protect the DNA of adult somatic cells and their telomeres continually shorten as they undergo rounds of cell division.

In 2010, scientists found that telomerase can reverse some age-related conditions in mice. These findings may contribute to the future of regenerative medicine. In the studies, the scientists used telomerase-deficient mice with tissue atrophy, stem cell depletion, organ failure, and impaired tissue injury responses. Telomerase reactivation in these mice caused extension of telomeres, reduced DNA damage, reversed neurodegeneration, and improved the function of the testes, spleen, and intestines. Thus, telomere reactivation may have potential for treating age-related diseases in humans.

Role in the cell cycle

Telomere shortening in humans can induce replicative senescence, which blocks cell division. This mechanism appears to prevent genomic instability and development of cancer in human aged cells by limiting the number of cell divisions. However, shortened telomeres impair immune function that might also increase cancer susceptibility.[28] If telomeres become too short, they have the potential to unfold from their presumed closed structure. The cell may detect this uncapping as DNA damage and then either stop growing, enter cellular old age (senescence), or begin programmed cell self-destruction (apoptosis) depending on the cell's genetic background (p53 status). Uncapped telomeres also result in chromosomal fusions. Since this damage cannot be repaired in normal somatic cells, the cell may even go into apoptosis. Many aging-related diseases are linked to shortened telomeres. Organs deteriorate as more and more of their cells die off or enter cellular senescence.