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EUKARYOTIC DNA REPLICATION

BY

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ZOOLOGY: SEM- V, PAPER- C11T: MOLECULAR BIOLOGY, UNIT 2: DNA REPLICATION

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Replication in Eukaryotes

Eukaryotic replication occurs during s-phase of cell cycle. Replication usually occurs only one time in a cell. Replication in eukaryotes occur in five stages namely,

1. Pre-initiation
2. Initiation
3. Elongation
4. Termination
5. Telomerase function

1. Pre-initiation:

Actually during pre-initiation stage, replicator selection occurs. Replicator selection is the process of identifying the sequences that will direct the initiation of replication and occur in G1 phase and occurs in G1 (prior to S phase). This process leads to the assembly of a multi protein complex at each replicator in the genome. Origin activation only occurs after cells enter S phase and triggers the Replicator – associated protein complex to initiate DNA unwinding and DNA polymerase recruitment. Replicator selection is mediated by the formation of pre-replicative complexes (pre-RCs). The first

step in the formation of the pre-RC is the recognition of the replicator by the eukaryotic initiator, ORC (Origin recognition Complex). Once ORC is bound, it recruits two helicase loading proteins (Cdc6 and Cdt1). Together, ORC and the loading proteins recruit a protein that is thought to be the eukaryotic replication fork helicase (the Mem 2-7 complex). Formation of the pre-RC does not lead to the immediate unwinding of origin DNA or the recruitment of DNA polymerases. Instead, the pre-RCs that are formed during G1 are only activated to initiate replication after cells pass from the G1 to the S phase of the cell cycle.

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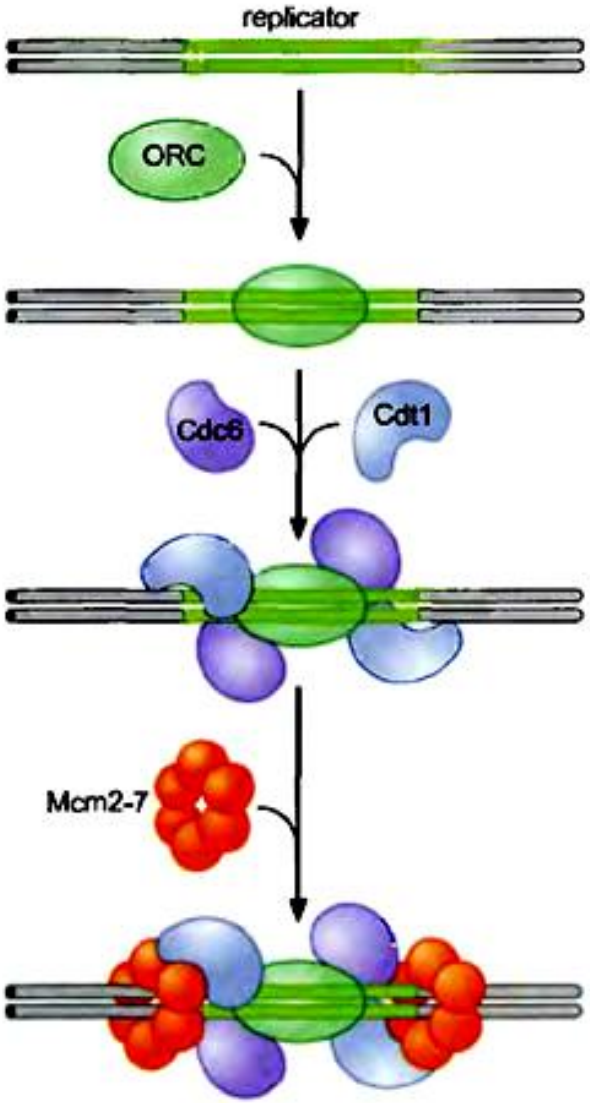

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2. Initiation:

Pre-RCs are activated to initiate replication by two protein kinases namely Cdk (Cyclin Dependant Kinase) and Ddk (Ddt4 Dependant Kinase). Kinases are proteins that covalently attach phosphate groups to target proteins. Each of these kinases is inactive in G1 and is activated only when cells enter S phase. Once activated, these kinases target the pre-RC and other replication proteins. Phosphorylation of these pro-proteins results in the assembly of additional replication proteins at the origin and the initiation of replication.

These new proteins include the three eukaryotic DNA polymerases and a number of other proteins required for their recruitment. Interestingly, the polymerases assemble at the origin in a particular order. DNA Pol d and e associate first, followed by DNA Pol a/primase. This order ensures that all three DNA polymerases are present at the origin prior to the synthesis of the first RNA primer (by DNA Pol a/primase). Once present at the origin, DNA Pol a/primase synthesizes an RNA primer and briefly extends it. Thus initiation of replication started.

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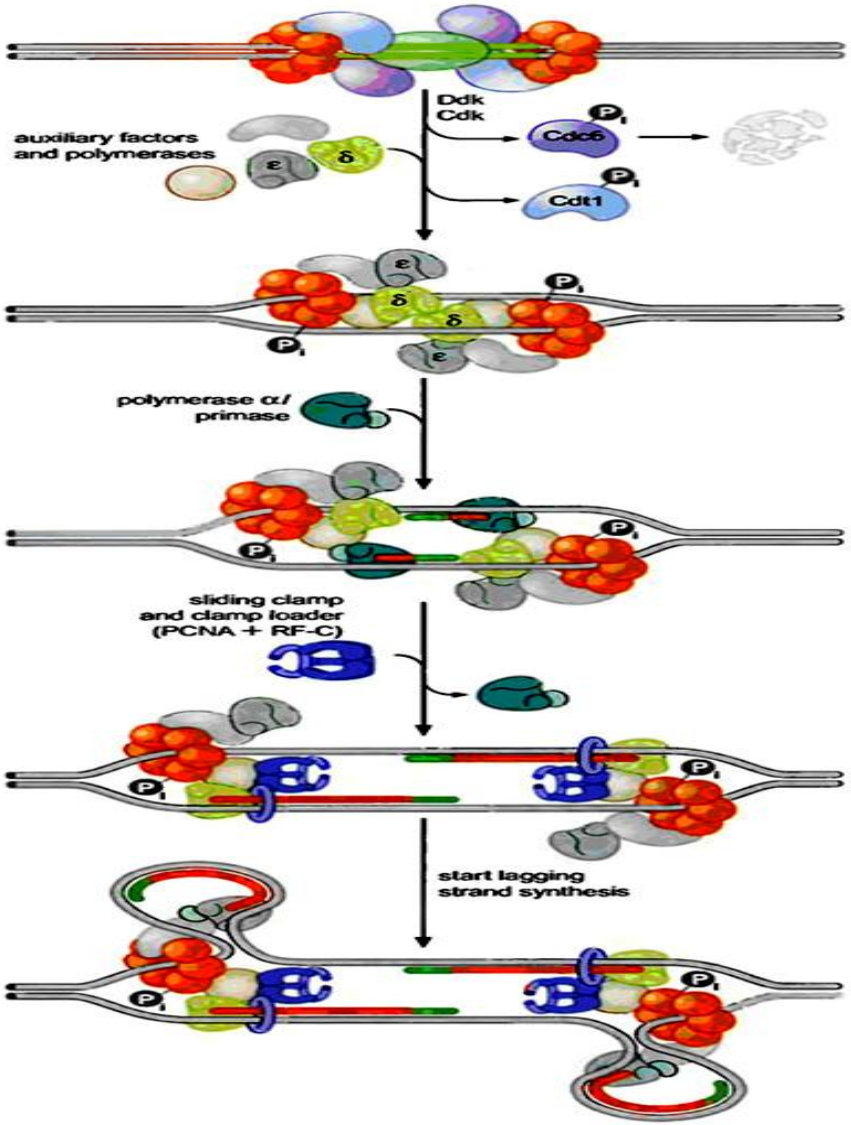

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3. Elongation:

The resulting primer-template junction is recognized by the eukaryotic sliding clamp loader (RF-C), which assembles a sliding clamp (PCNA) at these sites. Either DNA Pol d or e recognizes this primer and begins leading strand synthesis. After a period of DNA unwinding, DNA Pol a/primase synthesizes additional primers, which allow the initiation of lagging strand DNA synthesis by either DNA Pol d or e. In the diagram, Pol d was used for leading strand and Pol e was used for lagging strand synthesis. DNA Pol e possess

activity to remove primer and fills the gap with DNA like DNA Pol I in prokaryotes. SSB like activity was played by replication protein A (RP A) which is denoted as accessory factors during replication.

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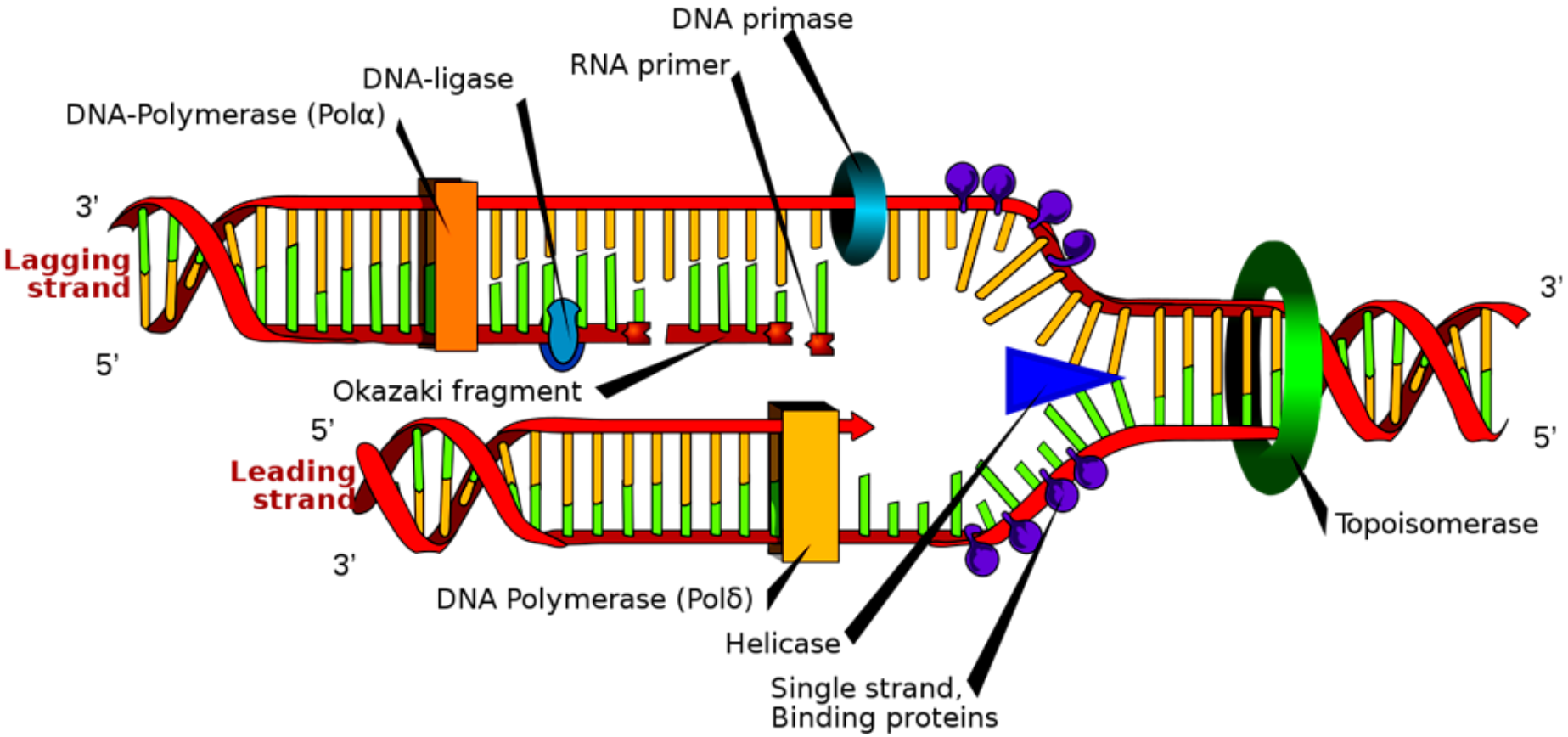

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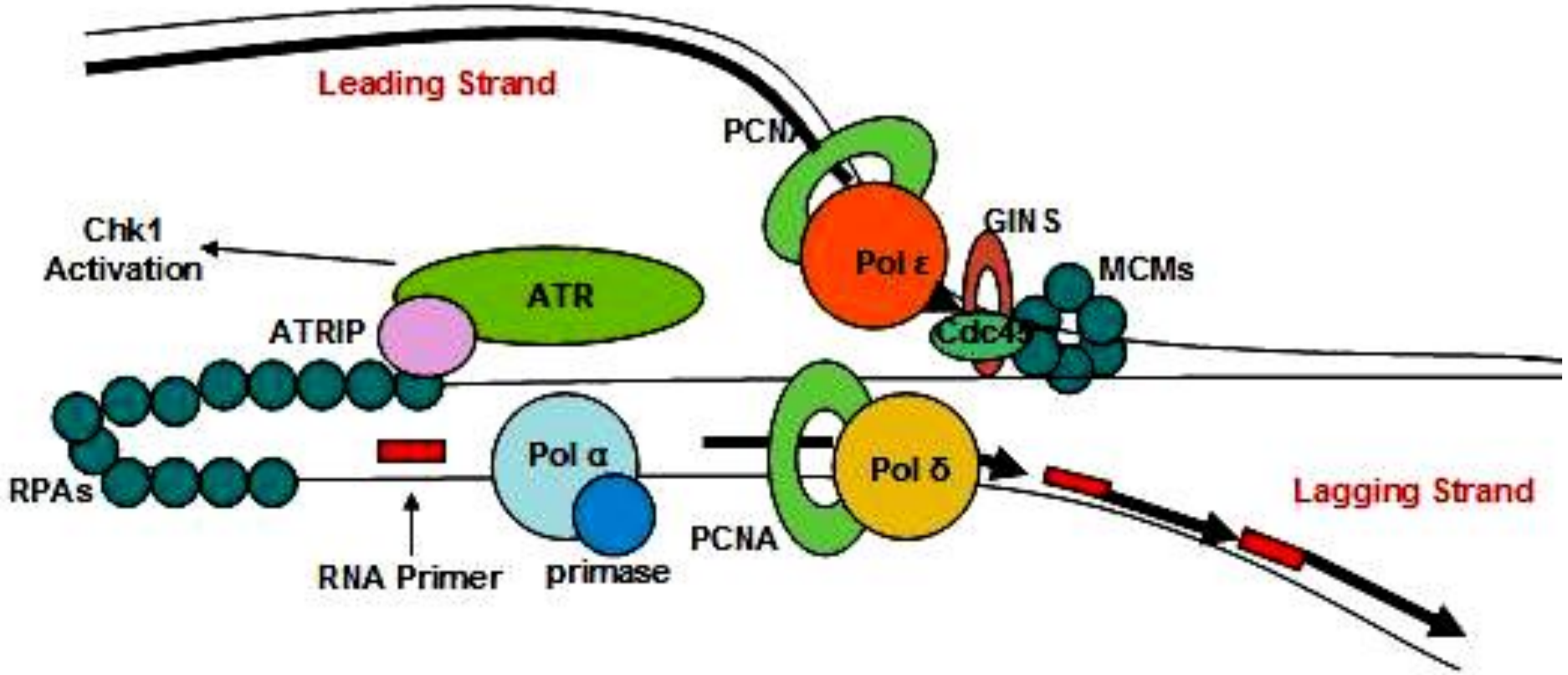

4. Termination:

When the replication forks meet each other, then termination occurs. It will result in the formation of two duplex DNA. Even though replication terminated, 5' end of telomeric part of the newly synthesized DNA found to have shorter DNA strand than the template parent strand. This shortage corrected by the action of telomerase enzyme and then only the actual replication completed.

5. Telomerase Function:

In Linear eukaryotic chromosome, once the first primer on each strand is remove, then it appears that there is no way to fill in the gaps, since DNA cannot be extended in the 3′–>5′ direction and there is no 3′ end upstream available as there would be in a circle DNA. If this were actually the situation, the DNA strand would get shorter every time they replicated and genes would be lost forever.

Elizabeth Blackburn and her colleagues have provided the answer to fill up the gaps with the help of enzyme telomerase. So, that the

genes at the ends, are conserved. Telomerase is a ribonucleoprotein (RNP) i.e. it has RNA with repetitive sequence. Repetitive sequence varies depending upon the species example *Tetrahymena thermophilia* RNA has AACCCC sequence and in Oxytrica it has AAAACCCC. Telomerase otherwise known as modified Reverse Transcriptase. In human, the RNA template contains AAUCCC repeats. This enzyme was also known as telomere terminal transferase.

The 3′-end of the lagging strand template basepairs with a unique region of the telomerase associated RNA. Hybridization facilitated by the match between the sequence at the 3′-end of telomere and the sequence at the 3′-end of the RNA. The telomerase catalytic site then adds deoxy ribonucleotides using RNA molecule as a template, this reverse transcription proceeds to position 35 of the RNA template. Telomerase then translocates to the new 3′-end by pairing with RNA template and it continues reverse transcription. When the G-rich strand sufficiently long, Primase can make an RNA primer, complementary to the 3′-end of the telomere's G-rich

strand. DNA polymerase uses the newly made primer to prime synthesis of DNA to fill in the remaining gap on the progeny DNA. The primer is removed and the nick between fragments sealed by DNA ligase.

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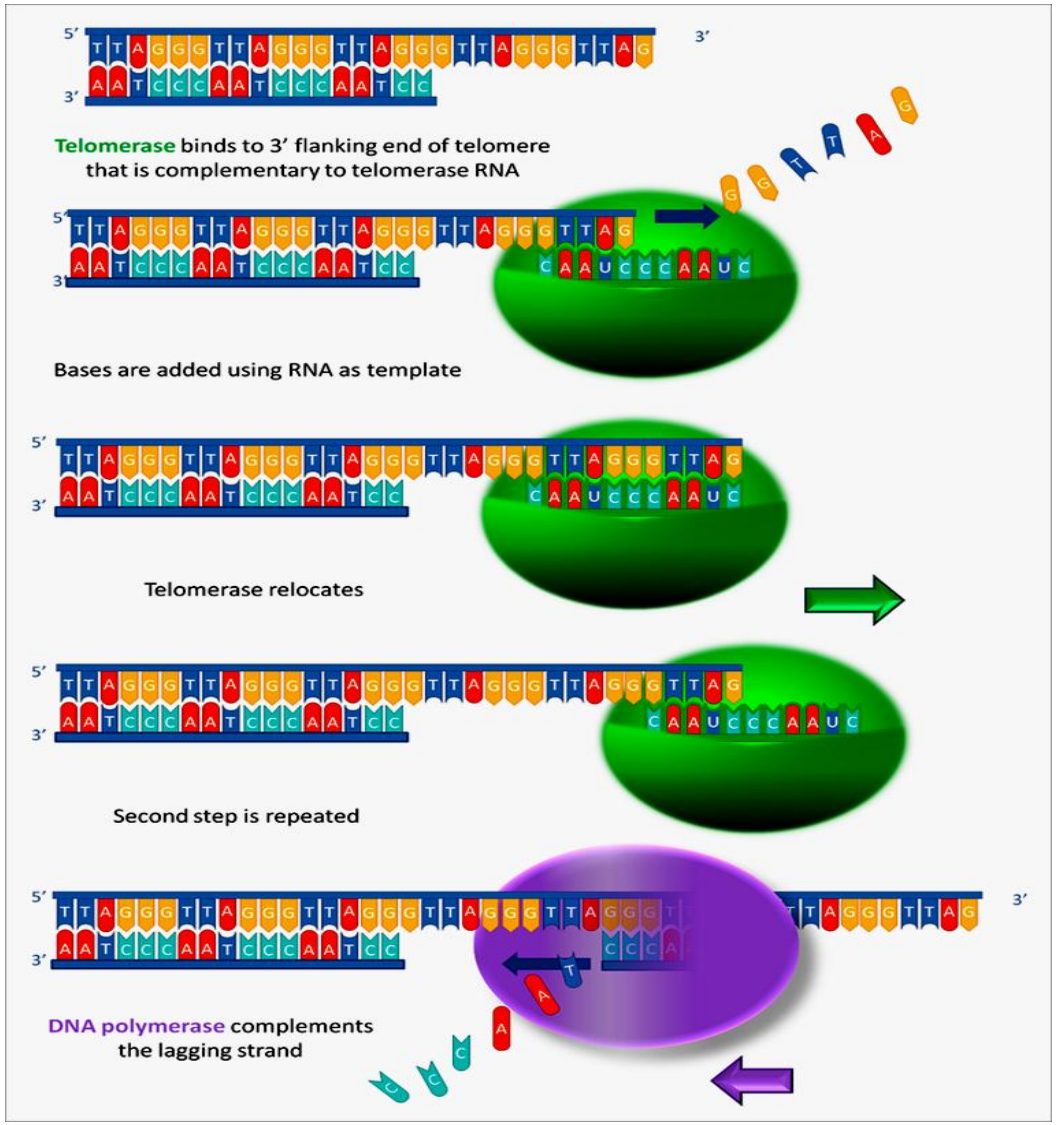

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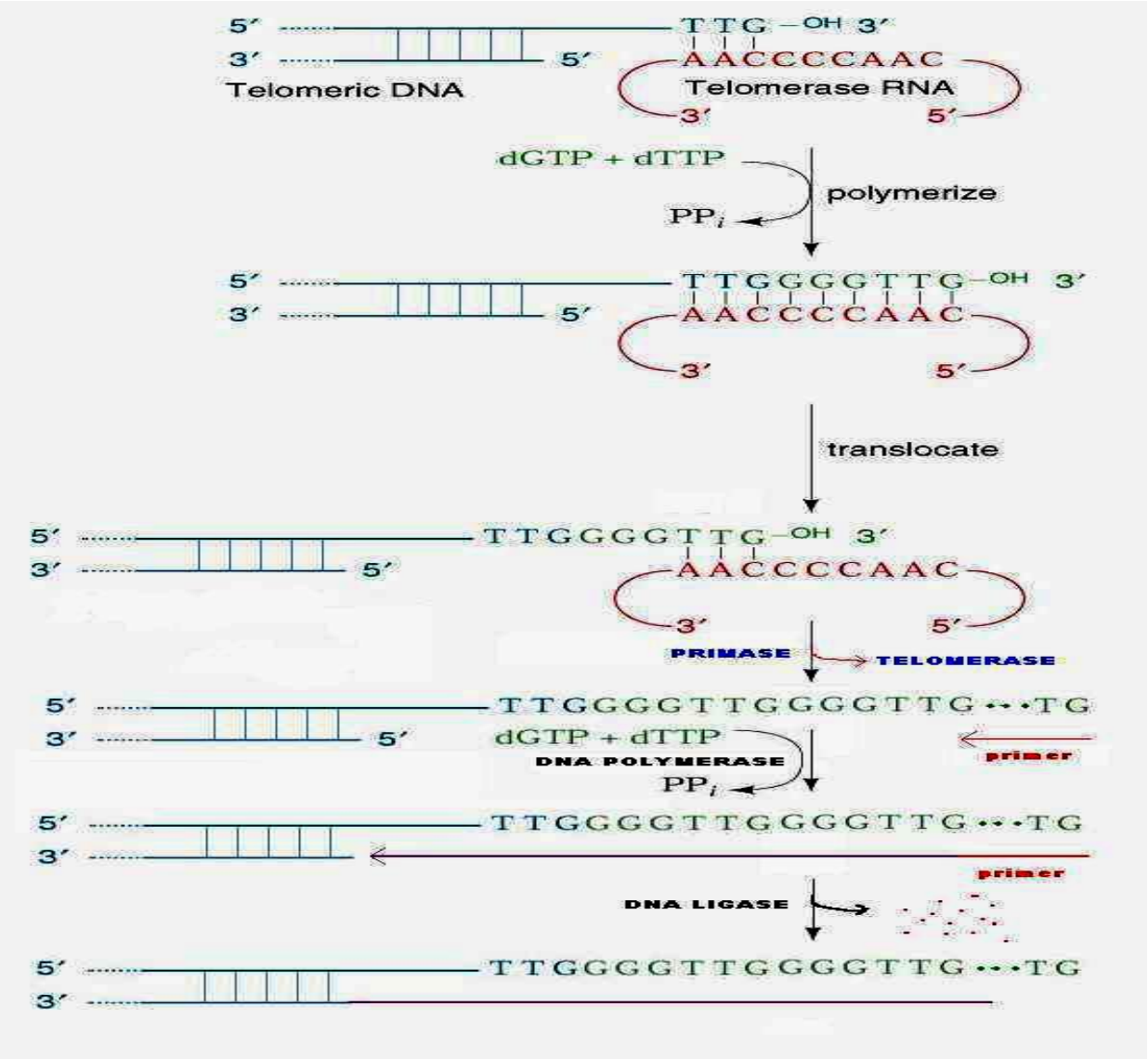

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THANK YOU

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