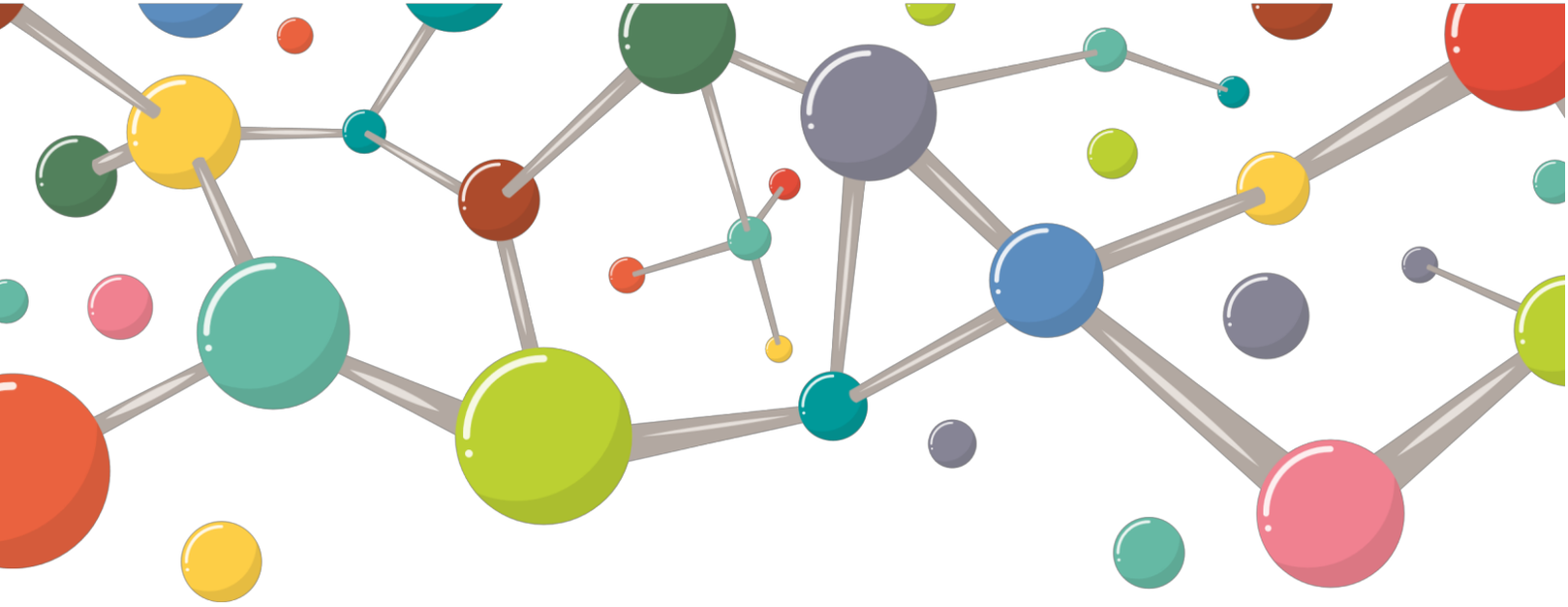

MOLECULAR BIOLOGY

S H E E T (5)

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Black color: doctor's explanation, Navy color: slides, Blue color: extra information, Red color: Dr. Khaldun's notes, highlighted sentence: modified, purple color: book notes .

General features of DNA Replication.

Semi-discontinuous Replication:

That means that one strand is synthesized in a continuous form, and the other strand issynthesized in a discontinuous form.
The continuous strand is called the: the Leading Strand.

The discontinuous strand is called the: The Lagging Strand.

This lagging strand is synthesized as short pieces of DNA, and each strand is called an Okazaki Fragment. And for each fragment there is one RNA primer (which means that thenumber of RNA primers is = to the number of Okazaki Fragments) while for the leading strand there has only one RNA Primer.

The gaps between the fragments after the RNA primers are removed (by H RNase) are filled by using DNA Polymerase I. And the last Phosphodiester bond is sealed (connected) by DNA Ligase.

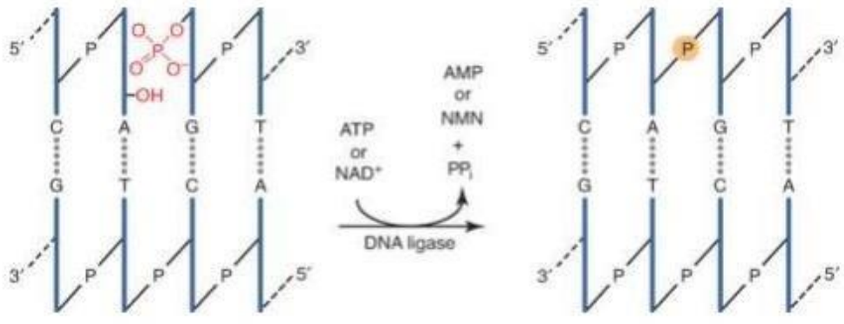


Figure 08.11: RNA primers for Okazaki fragments. The synthesis of Okazaki fragments is primed by short RNA segments.

The enzyme RNase H removes all of the primer's ribonucleotides at its 5' end (except the last one attached to the first deoxyribonucleotide), leaving a "gap" between two Okazaki fragments.

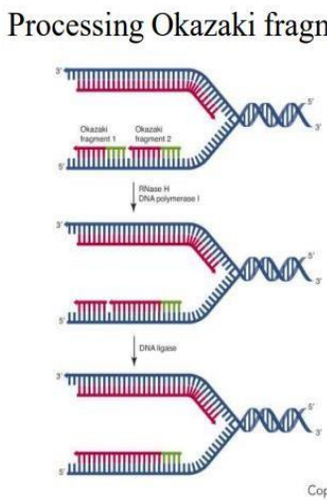


Figure 08.12: Pathway for processing Okazaki fragments.

In prokaryotic systems, DNA Polymerase I removes the last ribonucleotide of the primer (using its exonuclease part) and fills the entire gap between the fragments with deoxyribonucleotides using its polymerase part, replacing the original primer. And the same concept goes for Eukaryotic systems, but the protein is different (FEN I).

After the gap is filled by DNA pol I, DNA ligase is used to close off the "Nick" between the last deoxyribonucleotide added by DNA pol I and the deoxyribonucleotide after it.

Eukaryotic and prokaryotic systems have different proteins because Eukaryotes are more complex and require more complexed proteins. But prokaryotes are simpler so they require simpler proteins.

DNA polymerase I does its job by 3 enzymatic activities.

1. **It will remove the last ribonucleotide of RNA primers by 5`-3`**

   Exonuclease Activity.

2. **It will fill the gap formed by removing the original primer using its 5`-3` Polymerase Activity.**
3. **The last activity is used to correct the mistakes (proofreading) in DNA replication.**

The proofreading for the leading and lagging strands is done by DNA polymerase III because it has 2 activities.

1) **5`-3` Polymerase Activity.**
2) **3`-5` Exonuclease Activity.**

So, when any mistake happens through polymerization DNA Polymerase III will stop andcorrect the error.

But make no mistake, DNA Polymerase I Proofreads the DNA sequence that replaces the primer. But DNA Polymerase III proofreads the DNA connected to the primer. (Eukaryotic counterpart for DNA polymerase III is called DNA Polymerase δ)

(here the enzyme proofreads the sequence that it has built)

DNA replication is a complex process that can be divided into three stages. In this discussion, we will explore the key concepts of these stages.

First stage (Initiation):

The assembly of the replication apparatus at a unique site

-Replicon Model: 2 specific components:

Initiator protein and a replicator (a specific set of DNA sequences)

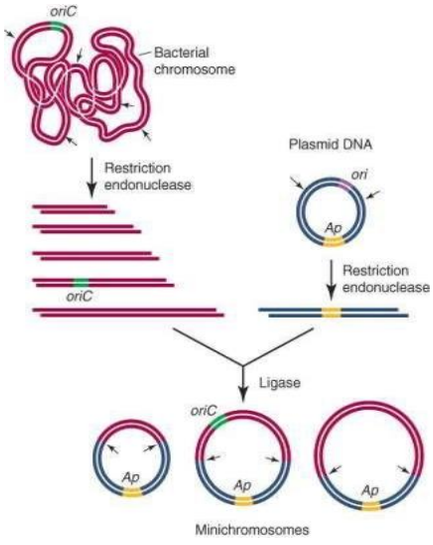
Both prokaryotic and eukaryotic cells have distinct sites where replication begins.

- **In prokaryotes, this site is known as the origin of replication (ori) and in the bacteria E.coli it's known as (oriC).**
- **Eukaryotes have multiple replication origins due to the large size of their genomes.**

To illustrate, let's consider the human genome, which consists of approximately 6 billion base pairs. Such a vast amount of DNA requires thousands or even hundreds of replication origins to complete replicationcycle within 20 hours. In contrast, the genome of E. coli, a prokaryotic organism, is much smaller, comprising only 5 million base pairs.
Consequently, E. coli can complete its replication cycle in just 20 minutes.

لو إنّك بكتيريا كان ما بتدرس موليكيولار وفوق هيك بتضاعف الجينوم تاعك بثلث ساعة!!

"وإذا الهُمومُ على الكِتافِ تَشعبطت
هُزّ الكُتوفَ وقُل لها يَختي .. انزَلي"



In the image provided, we can observe the specific site called the origin of replication initiation site.

This site consists of:

- **On the right side of ori C, there are approximately 5 repeats, and each repeat consists of 9 base pairs.**

  Five 9bp sites (R1-R5) are known as Dna A boxes.

- **On the left side, there are additional repeats, and each of these repeats is made up of 13 base pairs. Interestingly, these repeats on the left side are rich in adenine (A) and thymine (T) base pairs.**

  These repeats on the left side form a region known as the DNA Unwinding Element (DUE) with three 13bp AT-rich elements.

- **Multiple GATC sites that help in regulating DNA replication.**

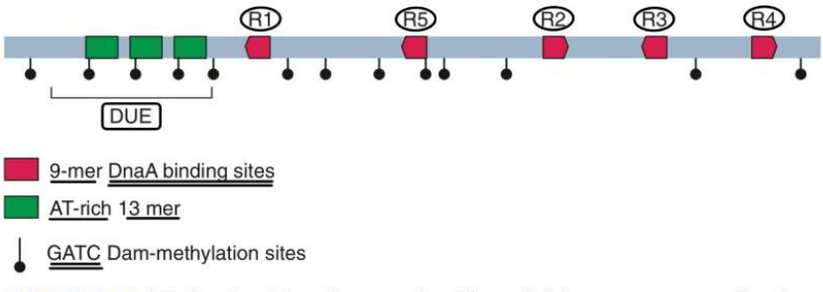
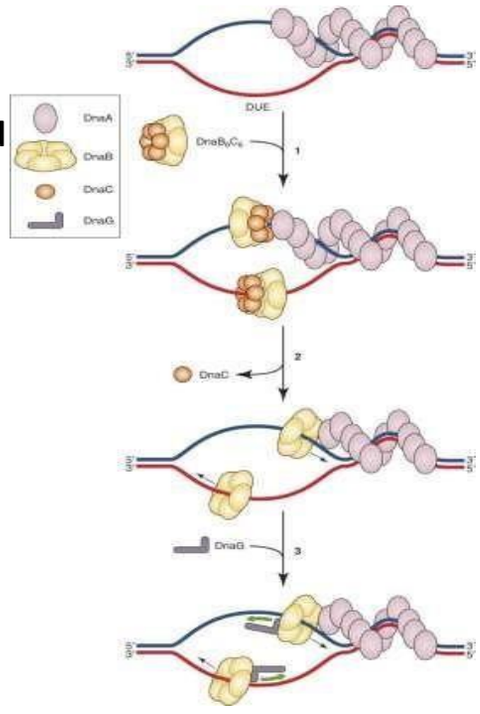


FIGURE 8.14 Minimal *oriC* region required for minichromosome replication.

During the initiation stage of DNA replication, initiator proteins play a crucial role. They recognize and bind to these specific sites, forming an initial complex. Subsequently, the initial complex transforms into an open complex. To further initiate and facilitate replication, additional proteins such as helicase and primase are recruited. Helicase assists in unwinding the double- stranded DNA, while primase helps in forming the priming complex necessary for replication to proceed.

Initiator proteins play essential roles during the initiation stage of DNA replication. The key initiator proteins include:

1. **DnaA (initiator): These proteins combine with ATP to form DnaA - ATP complexes, activating DnaA. These complexes then bind to the DnaA boxes (R1-R5) each of which are 9 bp's, to form what's known as a filament assembly. This filament assembly causes the AT-rich DEU region to melt (denature), initiating replication.**
2. **DnaB (helicase): This helicase protein is loaded onto the (unwound DUE region's strand) DNA strand by DnaC. It unwinds the DNA double helix, preparing it for replication.**
3. **DnaC (loader): As a loader protein, it assists in the assembly of thereplication machinery by helping DnaB bind to the DNA strand.**
4. **DnaG (Primase): It synthesizes RNA primers that serve as starting points for DNA polymerase during replication.**



"فَفِز بعلمٍ تَعِش حيًّا بهِ أبَدًا

فالناسُ موتى وأهلُ العِلمِ أحياءُ"

During the elongation stage of DNA replication, several enzymes and proteins are involved, act together at the replication fork, let's examine them:

1. **DNA polymerase I (Pol I): In prokaryotes, Pol I plays multiple roles. It mainly fills the gaps between Okazaki fragments with DNA, a process known as Okazaki fragment maturation. Additionally, Pol I is responsible for some error correction in the prokaryotic system. It possesses three activities: a 5'-3' polymerase activity, a 5'-3' exonuclease activity, and a 3'-5' exonuclease activity.**
2. **DNA Polymerase II (Pol II): Although not essential, Pol II also participates in DNA replication. Its specific functions are not well-defined.**

3. **DNA Polymerase III holoenzyme (Pol III): Pol III is the primary DNA synthesis enzyme during replication. It is responsible for synthesizing both the leading and lagging strands. DNA polymerase III holoenzyme has three distinct subassemblies**
    - **Core polymerase (previously known as pol III)**
    - **Clamp loader**
    - **Sliding clamp**

    Core polymerase possesses two essential activities and three subunits:
    - **α-subunit: a 5'-3' polymerase activity for synthesizing new DNA strands.**
    - **ε-subunit: 3'-5' exonuclease activity for proofreading and removing any incorrect nucleotides,**
    - **θ-subunit: Stimulates ε-subunit but not essential**
4. **Single-Stranded Binding Proteins (SSBs): These proteins bind to the single-stranded DNA regions exposed during replication. By stabilizing the single-stranded DNA, they prevent it from reannealing or forming secondary structures.**
5. **Topoisomerases (work together with DNA gyrase):**

These enzymes relieve the torsional stress and supercoiling that occur ahead of the replication fork. They accomplish this by cutting and resealing the DNA strands.

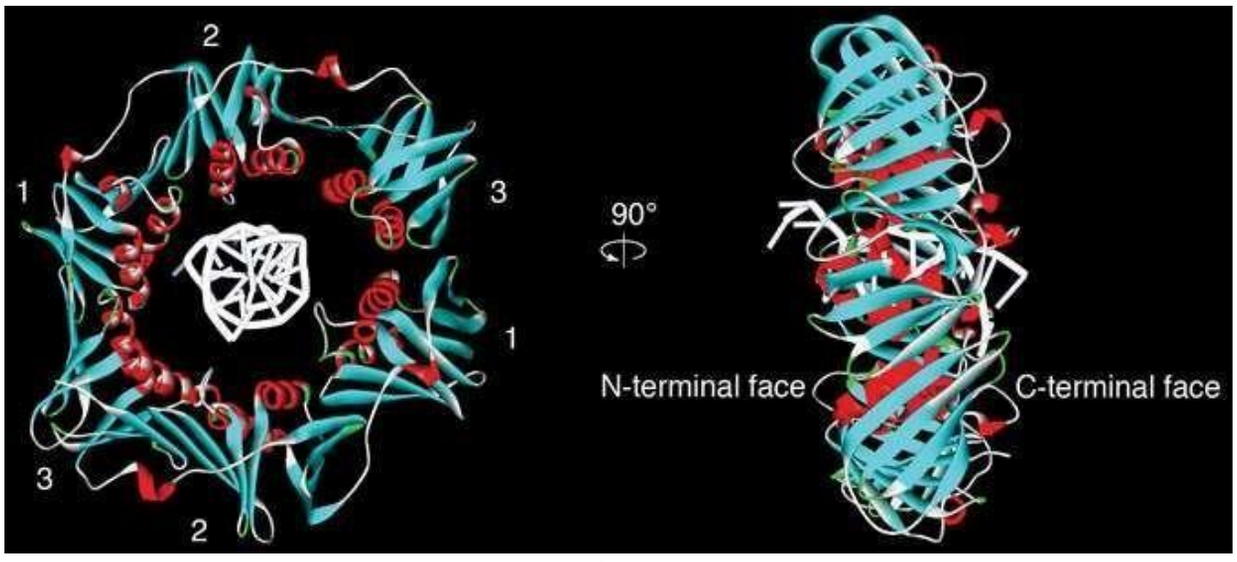
Replisome: DNA polymerase III holoenzyme, helicase (DnaB), primase (DnaG), single-stranded DNA binding protein.

Purified DNA polymerase III (Which is basically the core polymerase subassembly of the DNA pol III holoenzyme) is a very slow polymerase on its own because it dissociates from its DNA frequently. This means that purified DNA pol III enzymes have low processivity and dissociate from their template strand after adding only a few nucleotides.

Note: Enzymes that remain tightly associated with their template are said to be highly processive.

This problem is solved by adding the other subassemblies to form the complete DNA pol III holoenzyme, which is highly processive (even more processive than DNA pol I).

This is facilitated by the presence of a sliding clamp, a protein ring that prevents Pol III from dissociating. tethering the remainder of the polymerase holoenzyme to the DNA. The sliding clamp is loaded onto the DNA by the clamp loader complex, which requires ATP. This mechanism ensures continuous and efficient DNA synthesis during replication.

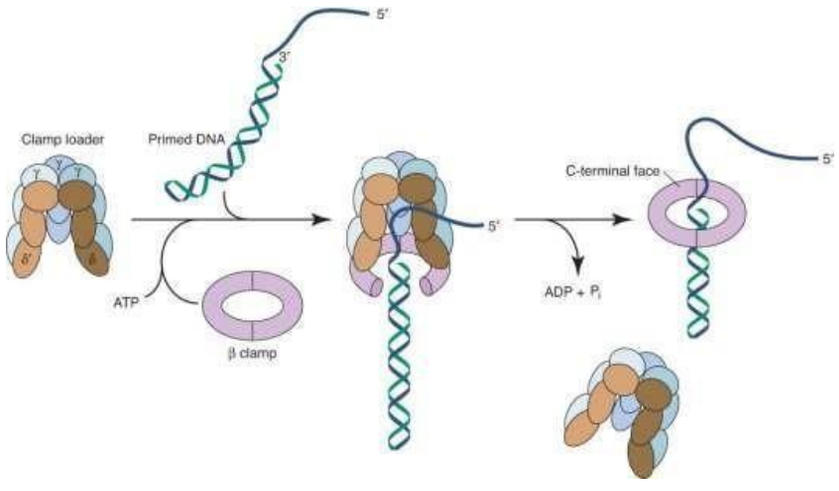


The structure of the sliding clamp

The clamp loader places the sliding clamp around DNA by using energy provided by ATP to load the sliding clamp onto a DNA template-primer with a 5' overhang.

DNA Polymerase I (Pol I) lacks a sliding clamp and a clamp loader, and therefore exhibits much lower processivity compared to the DNA Pol III holoenzyme (which means that this holoenzyme adds nucleotides at higher rate than DNA pol I).

It should be noted, however, that because DNA pol I lacks a clamp loader, it doesn't require ATP to function, in contrast to the holoenzyme.

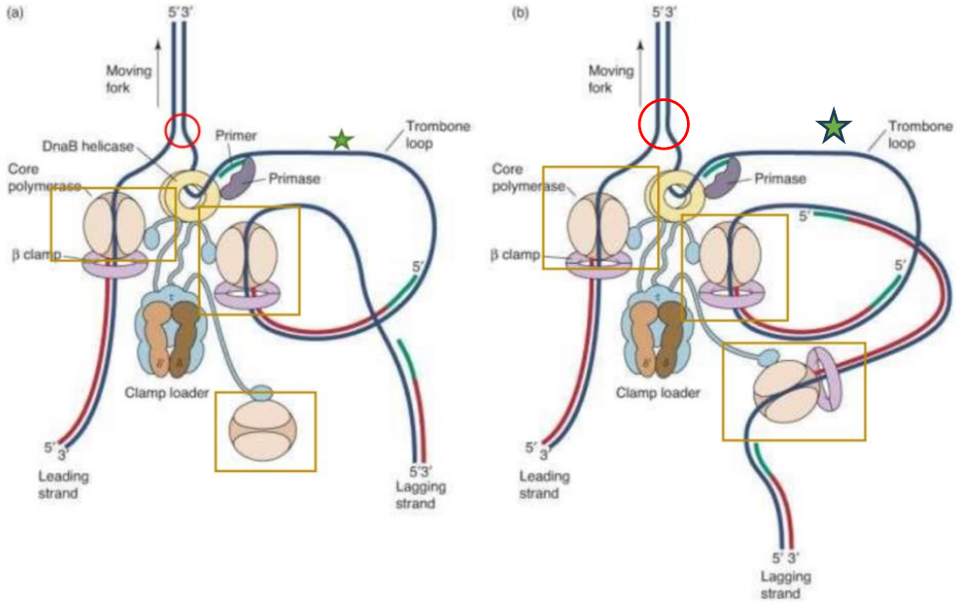


Experiments conducted on replicating bacterial DNA strands showed the the synthesis of the leading and lagging strands is coordinated at the replication fork. This discovery was very puzzling in the beginning because the core polymerase of each strand goes in opposite directions.

The Replisome explains how this coordination works, and one model of the replisome is the "Trombone model of replication". This model states that in order for the replisome to be able to coordinate the synthesis of the leading and lagging strands together, the lagging strand must loop out as each Okazaki fragment is formed, forming what's known as the "Trombone Loop".

The replisome consists of:

- **DNA Pol III holoenzyme**
- **Helicase**
- **Primase**
- **Single-stranded DNA binding protein.**



★ **Looping happens to allow the two polymerases to work at the same timeand to let each Okazaki fragment to extend in 5' to 3' direction.**

Each DNA pol III holoenzyme binds to three core polymerase via the clamp loader (because each clamp loader has 3 τ-subunits that bind to core polymerases). In situations where only one core polymerase is required for synthesizing the lagging strand, the third core protein will be off, just as it appears in the figure on the left.

When we unwind the double strand here we will make supercoils in the double strands that are ahead of them, and that will cause replication to pause until we use topoisomerases that relieve the supercoils in order to continue the replication.

- DnaB (helicase) must have a work speed that is greater or equal to the speed of additionof deoxynucleotides using DNA polymerase

- DNA polymerase adds 1000 nucleotides/second

- DnaB has a speed of 6000 round/minute

There are 3 steps for for the synthesis of bacterial Okazaki fragments of the lagging strand during elongation:

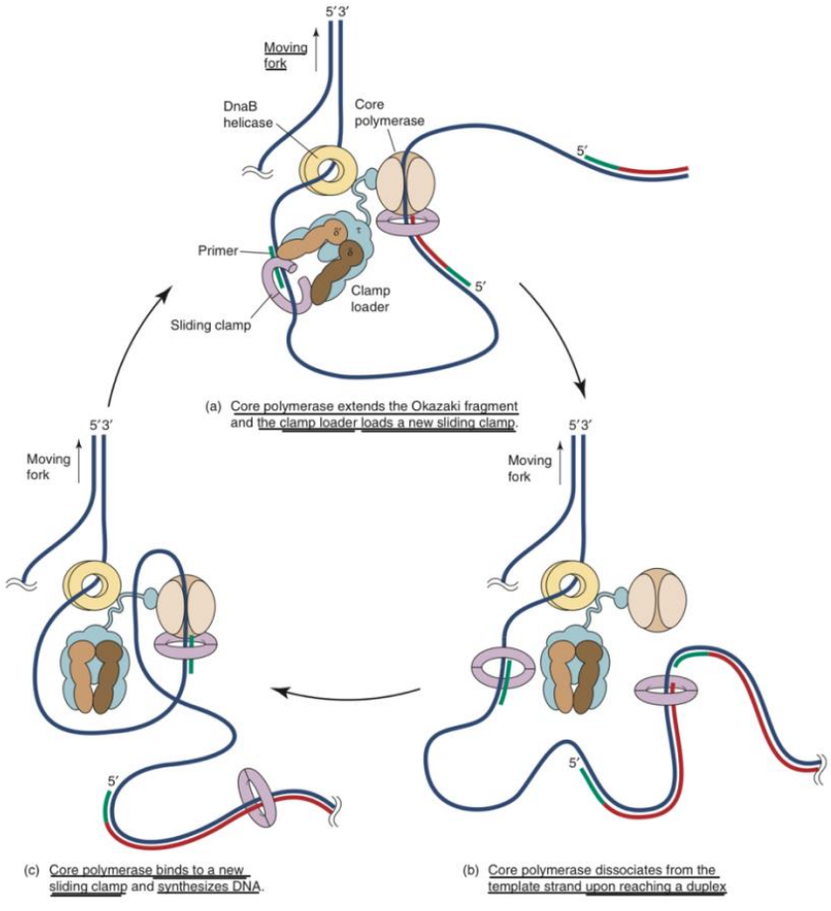
1. **Core polymerase extends the Okazaki fragment and clamp loader loads on a new sliding clamp.**

2. **Core polymerase dissociates from the sliding clamp upon reaching the previous Okazaki fragment that was synthesized before.**

3. **Core polymerase binds to the new sliding clamp and synthesizes DNA.**

The trombone loop is reset after each Okazaki fragment has been completed.

When Okazaki fragment synthesis is complete, the E. coli replication machinery uses two different release pathways to dissociate the core polymerase from its slide clamp. The first is known as "collision release", while the second is known as either "premature release" or "signaling release".



3) Termination:

E. coli DNA replication terminates when the two growing forks meet in the terminus region which is located 180 degrees around the circular chromosome from the origin.

The Terminus utilization substance (Tus) binds to the Termination site (Ter site).

Ter sites are conserved 11-bp sequences. E.coli has a total of 10 Ter sites:

-TerC, TerB, TerF, TerG, and TerJ block the progress of a replication fork that moves in a clockwise direction,

-TerA, TerD, TerE, TerI, and TerH block the progress in the opposite direction (counterclockwise)

The binding of Tus to Ter sites in the proper orientation arrests the progress of the replication fork by interfering with DnaB helicase's ability to unwind DNA.

Topoisomerase IV and recombinase separate newly formed sister chromosomes.

To finish replication correctly, the two forks eventually meet and join togetherwith the help of specific proteins. This allows the release of two circular DNA molecules called daughter chromosomes.

Once the daughter chromosomes are free, they need to be separated into individual cells. This separation is facilitated by an enzyme called recombinase, which breaks and rejoins the DNA strands through a processcalled recombination. This enzymatic action helps to separate the daughtercells.

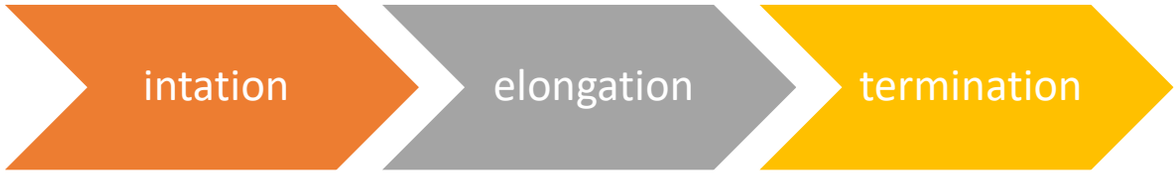
The termination stage of prokaryotic DNA replication involves specificcomponents: a termination site, topoisomerase IV and recombinase.

Eukaryotic DNA Replication

We begin our study of eukaryotic DNA replication by examining SV40 DNA replication because in vitro (artificial environment) studies of this system provide many important insights into eukaryotic DNA replication.

SV40 DNA is the genetic material of a virus (not a eukaryote) and is circular, double stranded, covalently closed, with only one origin of replication. But because the replication of this DNA occurs in eukaryotic cells, it share many of the properties and mechanisms that are attributed to eukaryotic DNA replication.

The same process



Initiation stage :

SV40 DNA replication requires some proteins to form the initiation replication complex (done by six T-antigen molecules which act as the initiator proteins and helicases in this case).

T-antigen and the eukaryotic cell's single-stranded DNA binding protein known as replication protein A (RPA), recruit DNA polymerase α-primase (Pol α), which is the only protein that can provide primase activity in eukaryotic cells.

The primase part of Pol α forms RNA primers 8-12 nucleotides long and after that it incorporates (constitutes) 15-25 deoxyribonucleotides (also known as initiator DNA) before dissociating from the template strand. This means that the polymerase part of Pol α is weakly processive and dissociates from DNA quickly.

Elongation stage :

Pol α works as both, a primase as it generates an RNA primer, and also works as a DNA polymerase that generates short DNA segments (the initiator DNA).

Additional proteins required for replication:

1. DNA Pol δ:

• Catalyzes leading and lagging strand synthesis

2. PCNA (proliferating cell nuclear antigen):

• The eukaryotic sliding clamp

3. Replication Factor C (RFC):

• Eukaryotic clamp loader

4. Flap endonuclease (FEN1):

• Acts during Okazaki fragment maturation, recognizes a double-stranded DNA with a 5′-unannealed flap and cleaves at the base of the flap.

***It works like DNA polymerase 1 in prokaryotes which removes the primers and fills the gaps**

5. DNA ligase:

• Seals the nick between adjacent Okazaki fragments using the same mechanism used in prokaryotes.

The RNA primer must be removed before DNA ligase joins the two fragments. This occurs with the help of FEN1 and RNase H.

All of these collaborate in the elongation stage of DNA replication.

This is a Trombone loop. ↘

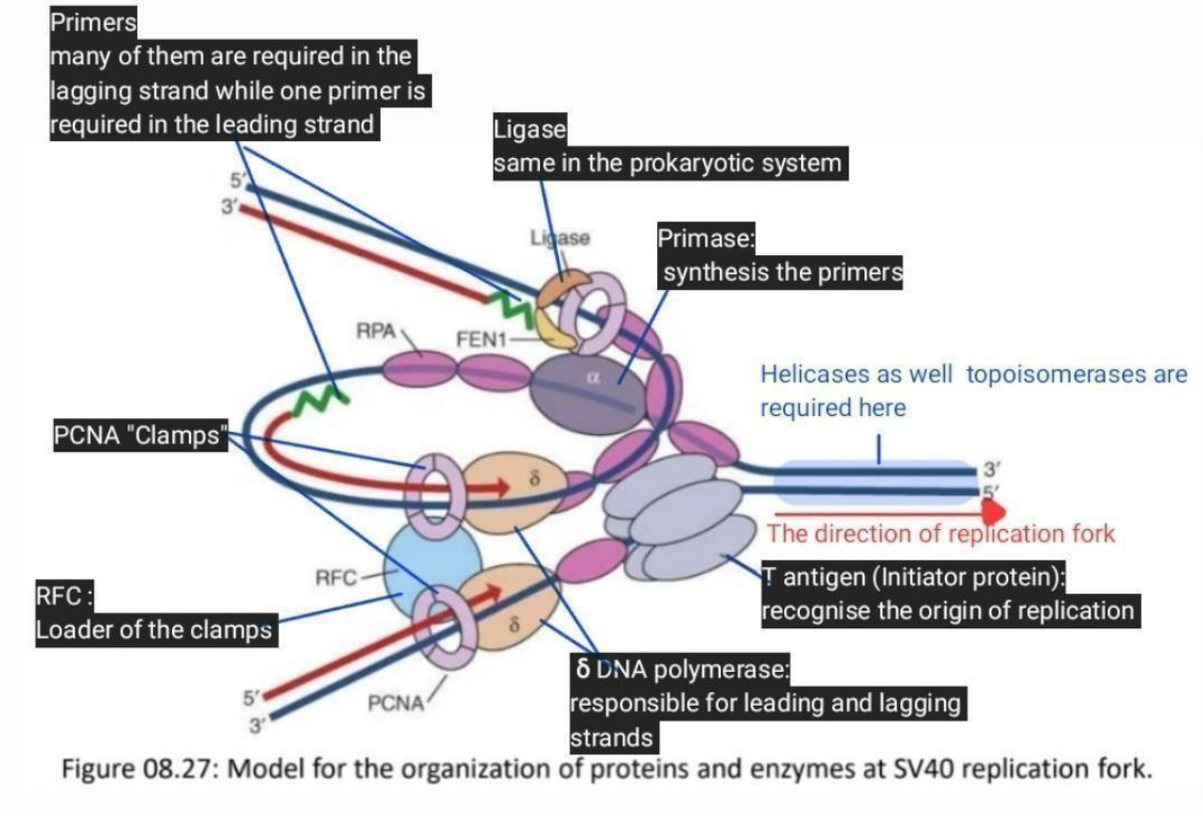


Figure 08.27: Model for the organization of proteins and enzymes at SV40 replication fork.

So far, the elongation process in eukaryotes is the same as in the prokaryotes, including the synthesis of lagging and leading strands and associating proteins, in both there is a need for ligases, single stranded DNA, primase, helicase, topoisomerase and polymerase.
It is important to point out that the polymerases of eukaryotic DNA only add 100- 200 deoxyribonucleotides per Okazaki fragment (which is 10% of the amount added in prokaryotes).

Up until now, we have been explaining how SV40 DNA replicates in eukaryotic cells using mainly eukaryotic proteins. This replication is very similar to the replication of actual eukaryotic DNA, but there are some differences. These differences arise because SV40 DNA is circular and has only one origin of replication, which is not the case in eukaryotic DNA, making the initiation and termination stages of the two types of replications different.

1. **Eukaryotic DNA replication initiates at multiple sites.**
2. **Multiple replication forks are formed and go in opposite direction (bidirectional).**
3. **Replication Forks continue till they fuse together.**
4. **Formation of new daughter DNA molecules.**

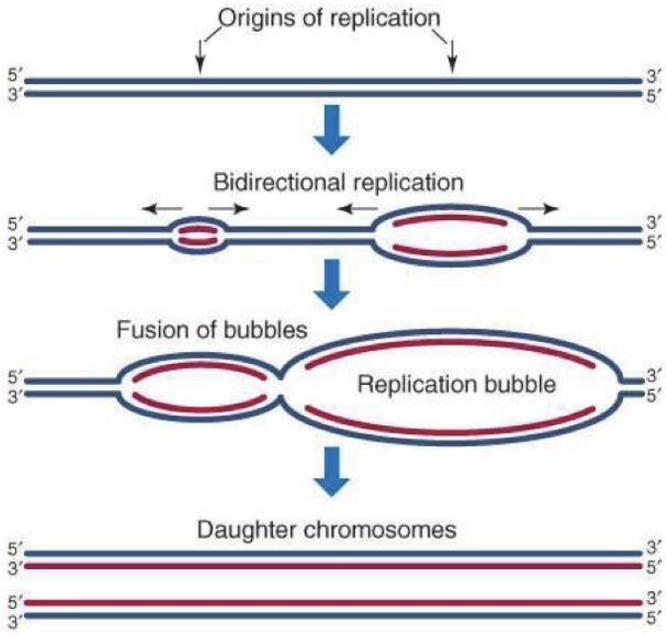


Figure 08.29: Schematic showing the fusion of a pair of replication bubbles.

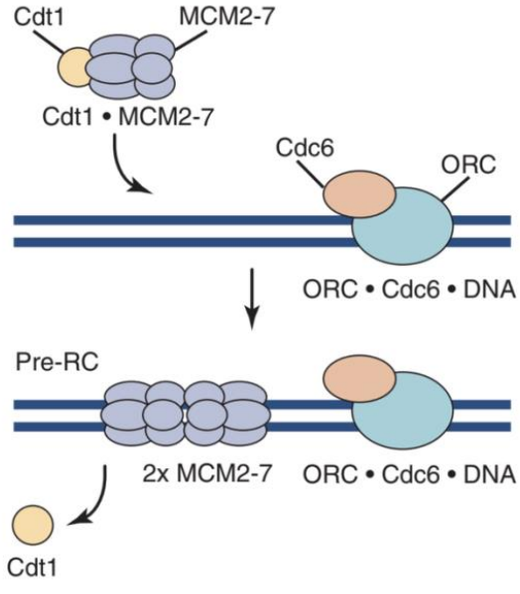
Eukaryotic chromosomes contain multiple origins of replication, and it must be ensured that each origin fires once, and only once, in each cell cycle.

Unlike SV40 DNA, which uses six T- antigen molecules to initiate replication, eukaryotic DNA replication initiation is facilitated by proteins known as Origin Recognition Complexes (ORCs). These proteins bind to eukaryotic origin sites and consist of six subunits, five of which bind ATP and have ATPase activity. ORC proteins serve as eukaryotic initiators.

In Yeast cells (which are eukaryotic), ORCs bind to DNA and recruit a cell division cycle protein known as Cdc6. This protein also binds ATP and has ATPase activity.

Cdc6 is essential for the initiation of yeast DNA replication and gets degraded once initiation is complete (to ensure that each origin of replication fires only one time during each cell cycle).

Two additional proteins are required to form the prereplication complex. These proteins, MCM2-7 (helicase) and Cdt1, combine to form a complex. MCM2-7 helicase, like bacterial DnaB helicase, has six subunits that each bind ATP and have ATPase activity.



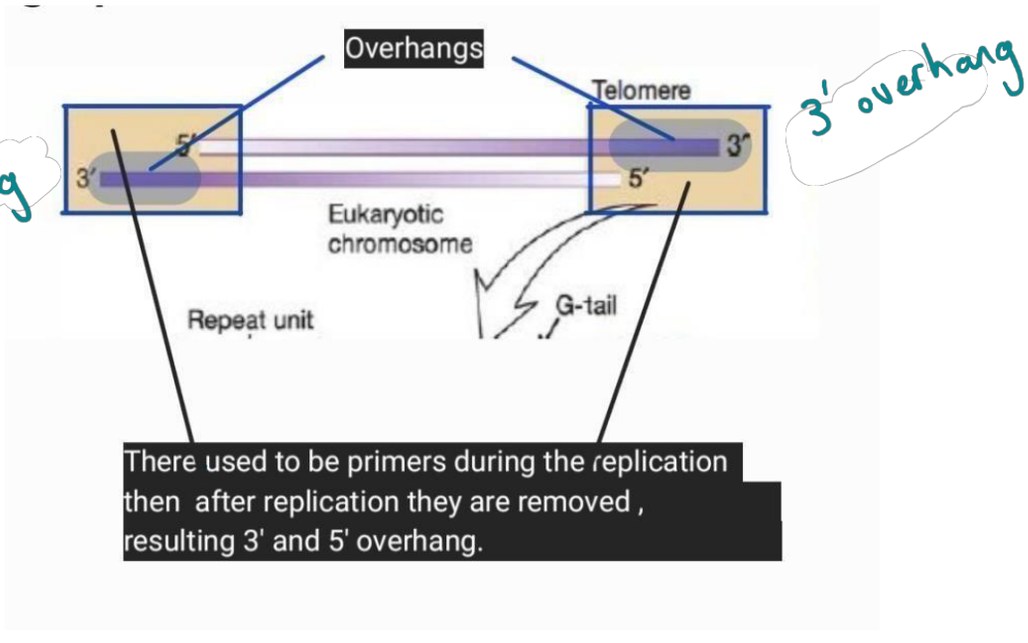
Termination stage:

Although some proteins are different, in SV40 DNA replication and the replication of eukaryotic, very similar actions occur during initiation and elongation. Termination however, is very different. This is because the eukaryote's genome is linear while in prokaryotes it's circular (such as the genome of SV40).
The termination is easier and less complicated in circular systems than in the linear DNA.
Few enzymes are required in prokaryotes like topoisomerase IV and recombinase.
Eukaryotic replication machinery on the other hand, must replicate long linear duplexes with multiple origins of replication that end with chromosomal telomeres.

- Eukaryotic chromosomes have multiple origins of replication.
- Require 3 different DNA polymerases.
- Linear eukaryotic chromosomes require telomerase to form their ends.
- The problem in eukaryotic system replication termination is the telomerase.

What makes the linear DNA termination different from circular DNA?

-The telomeres in the linear DNA.



During the replication of the linear double stranded DNA and removing of primers in the leading strand and the lagging strand it leaves gaps -at the ends-resulting overhangs.

*So if these telomeres are left like this there will be shortening of the chromosomes with each replication cycle by:
- Degradation by nucleases that chop these overhangs.
- The repeated replication cycles that cause losing of genes and digestion of chromosomes which leads to cell death.
*Why these regions cannot be replicated by normal DNA replication apparatus?
- There is no enzyme can polymerize in 3'-->5' direction using the overhangs as a template.

RECALL:

- Eukaryotic DNA polymerases work by extending a preformed primer and therefore cannot copy the very end of a linear duplex

There is a need for another mechanism to replicate these ends:

- It is called telomerasic system.

Telomerase: It is a terminal transferase-like enzyme which is required for telomere formation,it has 2 main features:

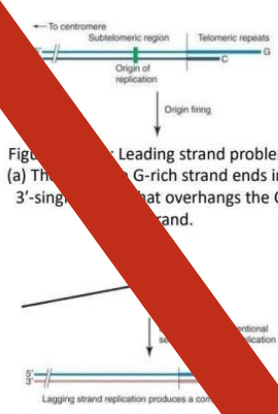
1. It acts as an RNA dependent DNA polymerase that supplies its own template RNA, How is that ?
- contains an RNA that acts as a template for telomeric DNA synthesis.

2. It has reverse transcriptase activity: which means that it uses RNA to synthesize DNA.

How?

—Telomerase uses an RNA template to add nucleotide repeats to the chromosome end

The leading strand replication presents a serious problem because it pro[illegible]s a blunt end with loss of sequence information, this occurs becau[illegible] replic[illegible] of the new G-rich strand will stop at the 5'-end of the short C[illegible] te[illegible]te strand, thus not producing a 3' overhang that is G-rich



Fig[illegible] Leading strand problem. (a) Th[illegible] G-rich strand ends in a 3'-sing[illegible]at overhangs the C-[illegible]rand.

Figure 08.37C: Leading strand p[illegible] Synthesis of lagging strands do[illegible] necessarily present a problem fo[illegible] replication machinery.

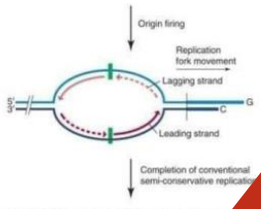


Figure 08.37B: Leading strand pr[illegible] (b) Semiconservative replicatio[illegible] at origins internal to the t[illegible] repeats.

Figure 08.37D: Le[illegible]d problem. (d) Synthesis of l[illegible]nds introduces a problem bec[illegible]tion stops at the 5'-e[illegible]rich template.

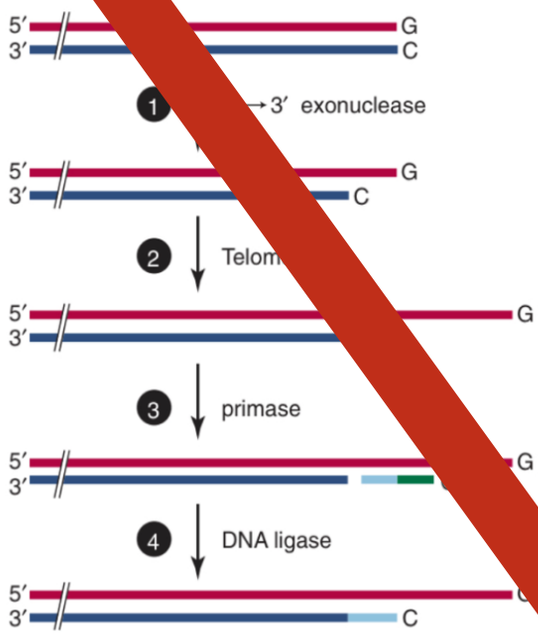
Telomerase plays an important role[illegible]ing the End-replication problem:

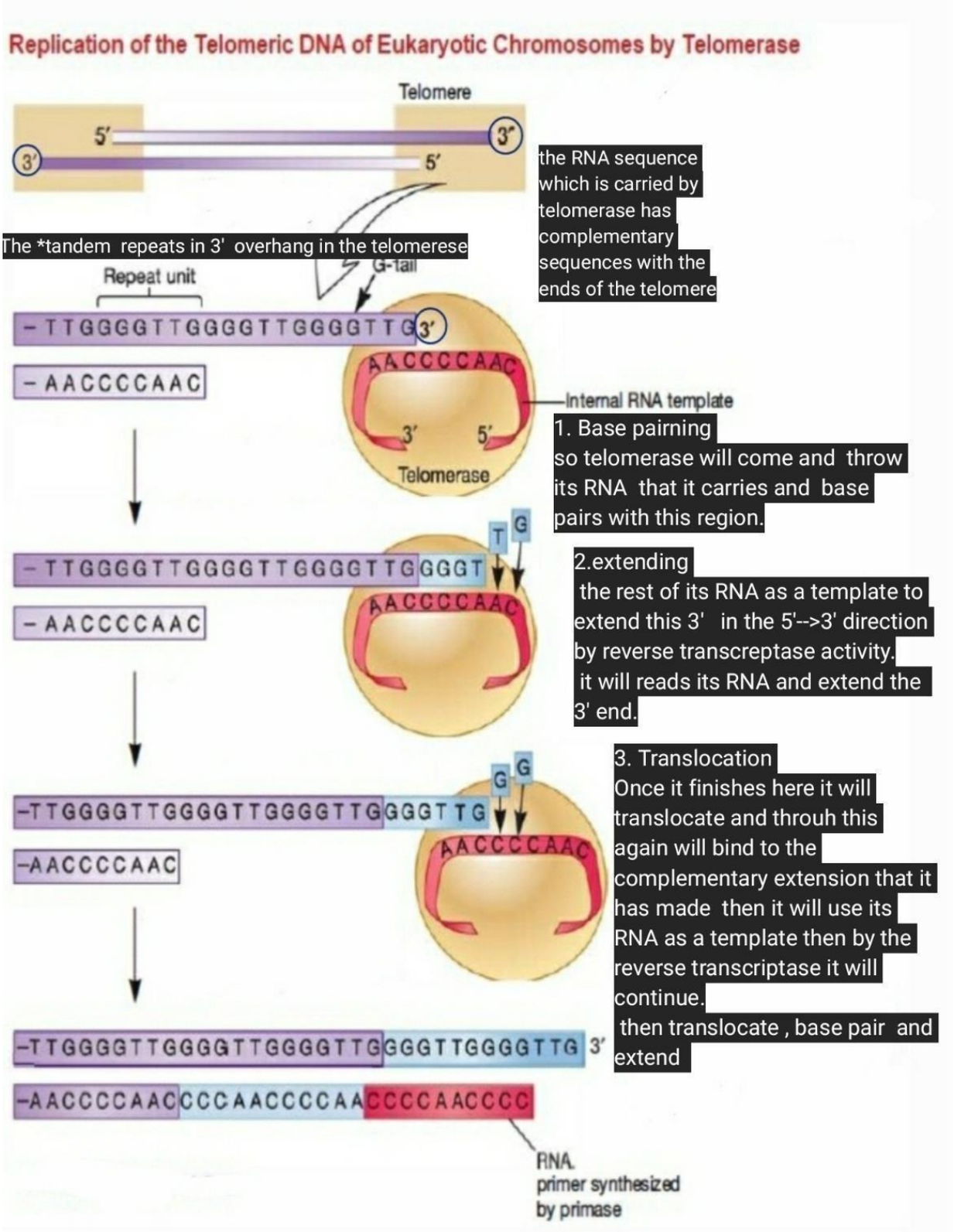
Telomerase participates in solving th[illegible] by modifying the blunt ends that resulted from the primary DNA re[illegible]io[illegible]s modification goes as follows:

1. **A strand specific DNA 5'→3' e[illegible]clease remo[illegible]nucleotides to generate an overhang.**

2. **Telomerase extends th[illegible] overhang.**

3. **Newly synthes[illegible]region serves as a template f[illegible]tandard replication, restoring[illegible]5' end.**





When the single strand of the telomere is long enough, primase can synthesize a primer, and DNA polymerase can extend the primer with DNA and then the ligase will seal the nick

FIGURE NOTES:

No.1: telomerase Adds the same sequence at the 3 ' overhang because it uses thesame template of RNA which is the telomere repeated tandem repeats of GGGTTG

No.2:*A tandem repeat is a sequence of two or more DNA bases that is repeatednumerous times in a head-to-tail manner on a chromosome

No.3: the primase binds to the new extended part of 3' hangover, and synthesizethe primer according to the template strand in 5'to 3' direction, after that the primer will be removed.

No.4: telomeres always have 3' overhang.

In this way, the telomeres are extended to large extent in each replication cycle, however, the shortening still existed in the telomeres but without reaching the internal genes.

The following notes are just for revision:

Recall:

***In somatic cells*: there is no active or partially active telomerase, so the region oftelomeres at the end of chromosomes is not going to be replicated (partially shortening) and there will be continuous shortening in both ends of the chromosome and the genes from both sides will be lost, over time the cell will die, and that is one cause of aging process.**

NOTE:

aging process is related to the length of telomeres and with the activity of telomerase (If telomerase is not active, telomeres will be shorter and finally thecell will die)

In cancer cells

The telomerase is very active always replicating the ends preventing the death ofthe cells.

In the embryonic cells

the telomerase is very active so always extending telomerase and the cells produceand devide continuously without shortening in the ends of chromosomes.

*In eukaryotes the DNA is covered with nucleosomes. DNA replication can't be done while those nucleosomes are covering the DNA because DNA replication requires a lot of proteins that must interact with the DNA , so those
nucleosomes must be disassembled and then the DNA will be exposed to DNA replication to bind and the replication can go ahead. After it has been replicated, the nucleosomes will be re-assembled again.
**Note that this doesn't happen in prokaryotes because there is no nucleosome associated.
Now how to disassociate this tones from nucleosomes?
-By adding acytel, methyl, phosphate or other groups to histones and by some enzamytic reactions histones will lose their (+) positive charges and disassociate.

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