RNA Priming
&
Replication of Telomeres

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As the two strands separate or unzip, the bases are exposed, and the enzyme *DNA polymerase II* moves at the point where synthesis will begin. The short segment of RNA that provides the necessary 3´ OH terminus for the initiation of *DNA polymerase* activity is called a **primer**. The primer is laid down complementary to the DNA template by an enzyme *RNA polymerase* or *Primase*. Hence we can say that synthesis of leading strand begins at the origin of replication is initiated by a *primase* (*RNA polymerase*). The DNA replication requires a template DNA strand to copy and a primer strand to which nucleotides can be added. The enzyme DNA *polymerase s*ynthesizes DNA in a 5´→3´ direction. The short RNAs synthesized by the *primase* at the 5´ end of the leading strand and the 5´ end of the each Okazaki fragment serve as the primer for the synthesis of DNA by *DNA polymerase*.

In bacteria, *primase* and *helicase* associate to form a protein complex called primosome. Primosome is involved in the synthesis of RNA primer sequences used in DNA replication. It moves along a DNA molecule with the help of energy of ATP. The *helicase* moves along the lagging strand template, while *primase* binds to helicase periodically and synthesizes short RNA primers that begin the formation of Okazaki fragments. The discovery that DNA gets synthesized as short fragments due to discontinuous replication was made by Reiji Okazaki. The initiation of Okazaki fragments on the lagging strand is carried out by primosome (Fig.11.9). As it proceeds, the *DNA helicase* unwinds the parental DNA double helix, and synthesizes RNA primers needed for the discontinuous synthesis of the lagging strand. *DNA primase* synthesizes RNA primers that are covalently extended with the addition of deoxyribonucleotides by *DNA polymerase III*. Single stranded DNA binding protein coats the unwound unreplicative DNA and keeps it in an extended state for *DNA polymerase* III. The RNA primers are replaced with DNA by

DNA polymerase I and the single strand nicks left by *polymerase I* are sealed by DNA *ligase.* The complete replication apparatus moving along the **Dnb A** molecule at replication fork is called **replisome.** The replisome contains *DNA polymerase* III holoenzyme, two catalytic cores one replicating the leading strand and the other replicating the lagging strand.

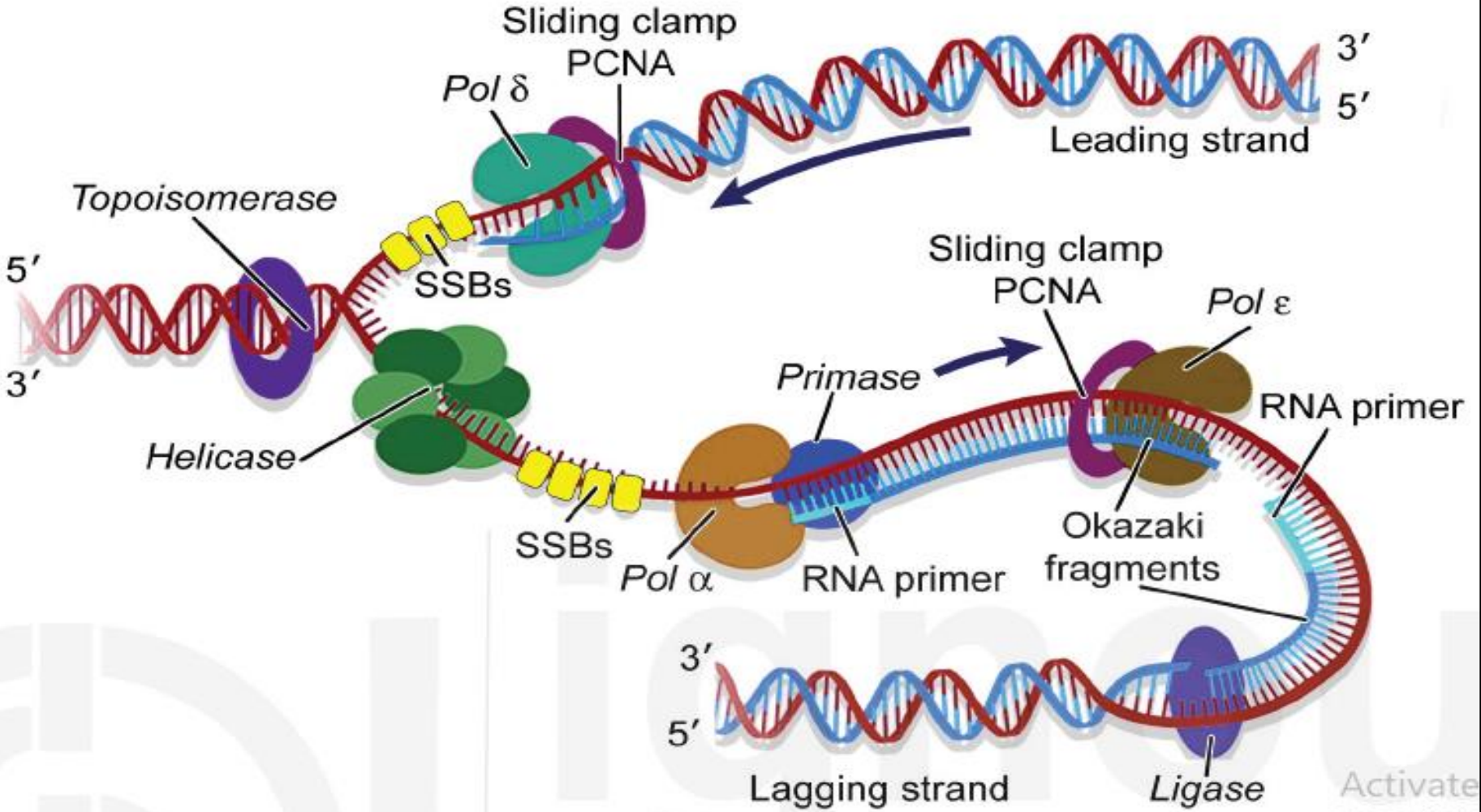


Fig. 11.9: Diagram showing priming during DNA replication in prokaryotes.

TELOMERS AND TELOMERASE

DNA REPLICATION

- DNA replication is fundamental process occurring in all living organism to copy their DNA. The process is called replication in sense that each strand of ds DNA serve as template for reproduction of complementary strand.
- DNA replication is semiconservative. Each strand in the double helix acts as a template for synthesis of a new, complementary strand.
- New DNA is made by enzymes called DNA polymerases, which require a template and a primer (starter) and synthesize DNA in the 5′ to 3′ direction.
- During DNA replication, one new strand (the leading strand) is made as a continuous piece. The other (the lagging strand) is made in small pieces.
- DNA replication requires other enzymes in addition to DNA polymerase, including DNA primase, DNA helicase, DNA ligase, and topoisomerase.

- DNA Replication initiates at a single site in Prokaryotes and at multiple sites in Eukaryotes.

- As compared to eukaryotes, nucleotide addition during DNA replication occurs almost 20 times faster in prokaryotes.

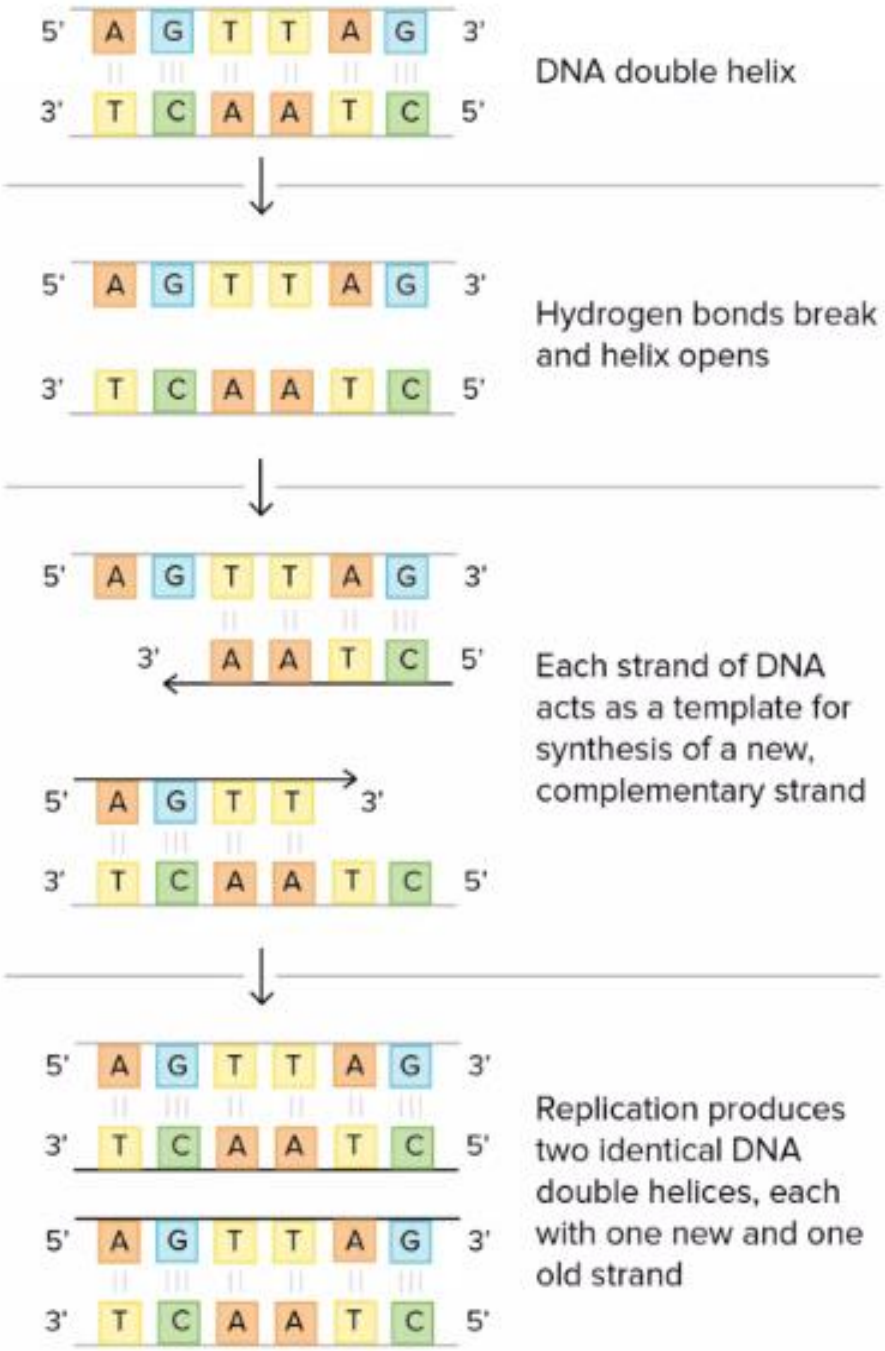
- Location:

Prokaryotes do not have nucleus and other membrane-bound organelles, like mitochondria, endoplasmic reticulum, and Golgi bodies. The prokaryotic DNA is present as a DNA-protein complex called nucleoid. The replication occurs in the cytoplasm of the cell.

In case of eukaryotes, the organisms that contain a membrane-bound nucleus, the DNA is sequestered inside the nucleus. Hence, the nucleus is the site for DNA replication in eukaryotes.

➢ Stage of Cell Division:

- ✓ In prokaryotes, DNA replication is the first step of cell division, which is primarily through binary fission or budding.
- ✓ In eukaryotes, cell division is a comparatively complex process, and DNA replication occurs during the synthesis (S) phase of the cell cycle.

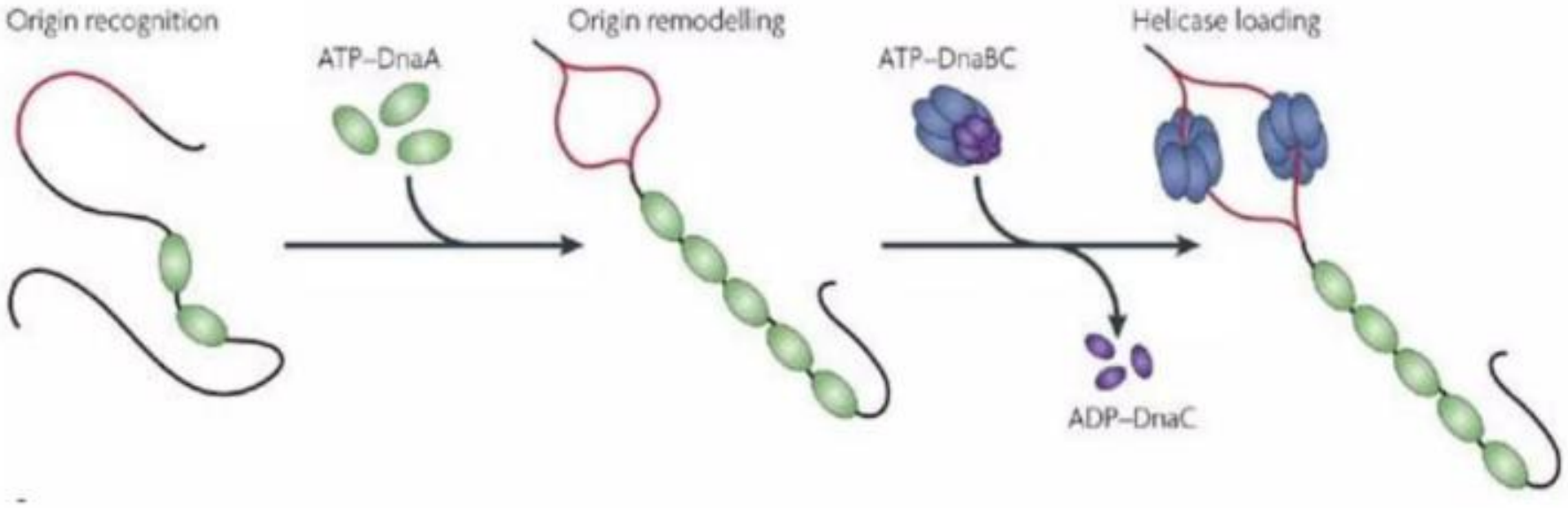


- **Initiation:** DNA replication is initiated at a specific or unique sequence called the origin of replication, and ends at unique termination sites. The region of DNA between these two sites is termed as a replication unit or replicon.

- **Prokaryotic DNA** is organized into circular chromosomes, and some have additional circular DNA molecules called plasmids. The prokaryotic DNA molecules contain a single origin of replication and a single replicon. Moreover, these origin sites are generally longer than eukaryotic origin sites.

- **Eukaryotic DNA** is comparatively very large, and is organized into linear chromosomes. Due to the high amount of material to be copied, it contains multiple origins of replication on each chromosome. DNA replication can independently initiate at each origin and terminate at the corresponding termination sites. Thus, each chromosome has several replicons, which enable faster DNA replication. The human genome that comprises about 3.2 billion base pairs gets replicated within an hour. If DNA replication was dependent on a single replicon, it would take a month's time to finish replicating one chromosome.

Initiation of DNA Replication in *E. coli*



DnaA binds to high affinity sites in oriB

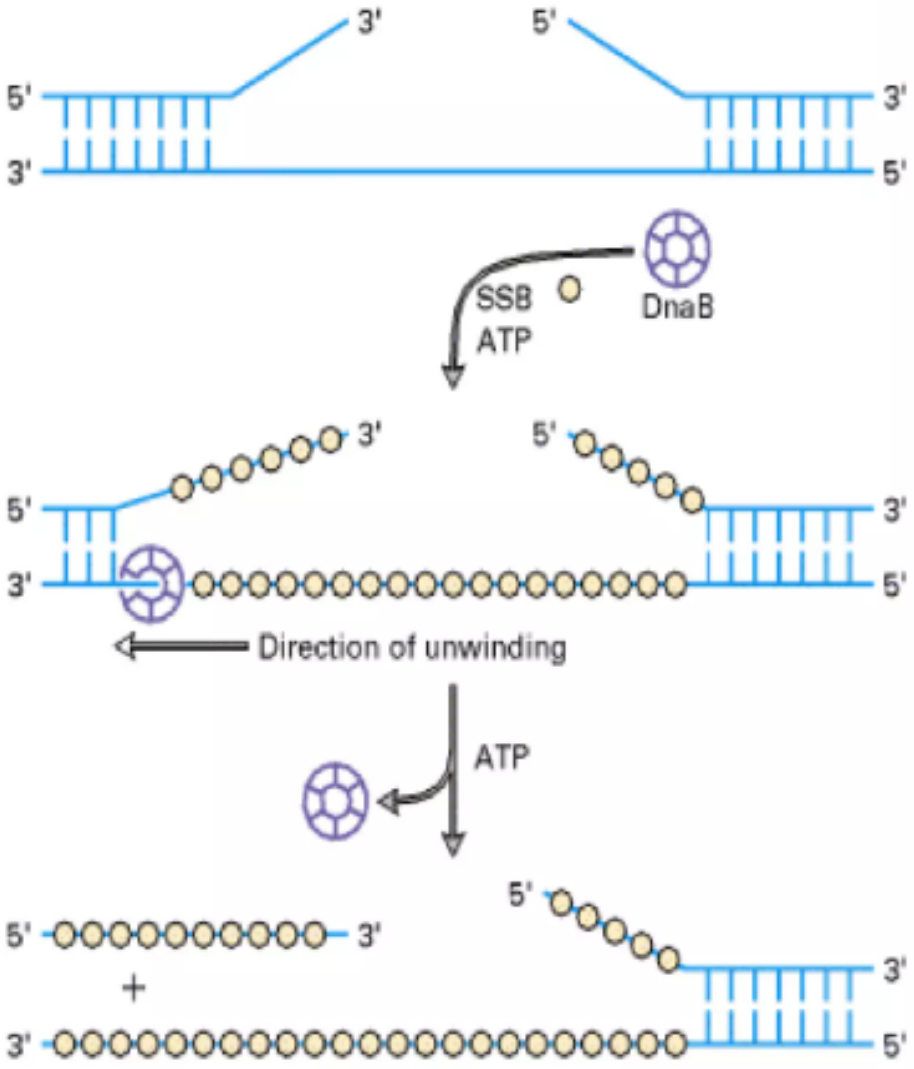
DnaA facilitates the melting of DNA-unwinding element

DnaC loads DnaB helicase to single stranded regions

DnaB helicase unwinds the DNA away from the origin

from Mott and Berger, *Nature Rev.Microbiol.* **5**, 343 (2007)

DnaB is an ATP-dependent Helicase



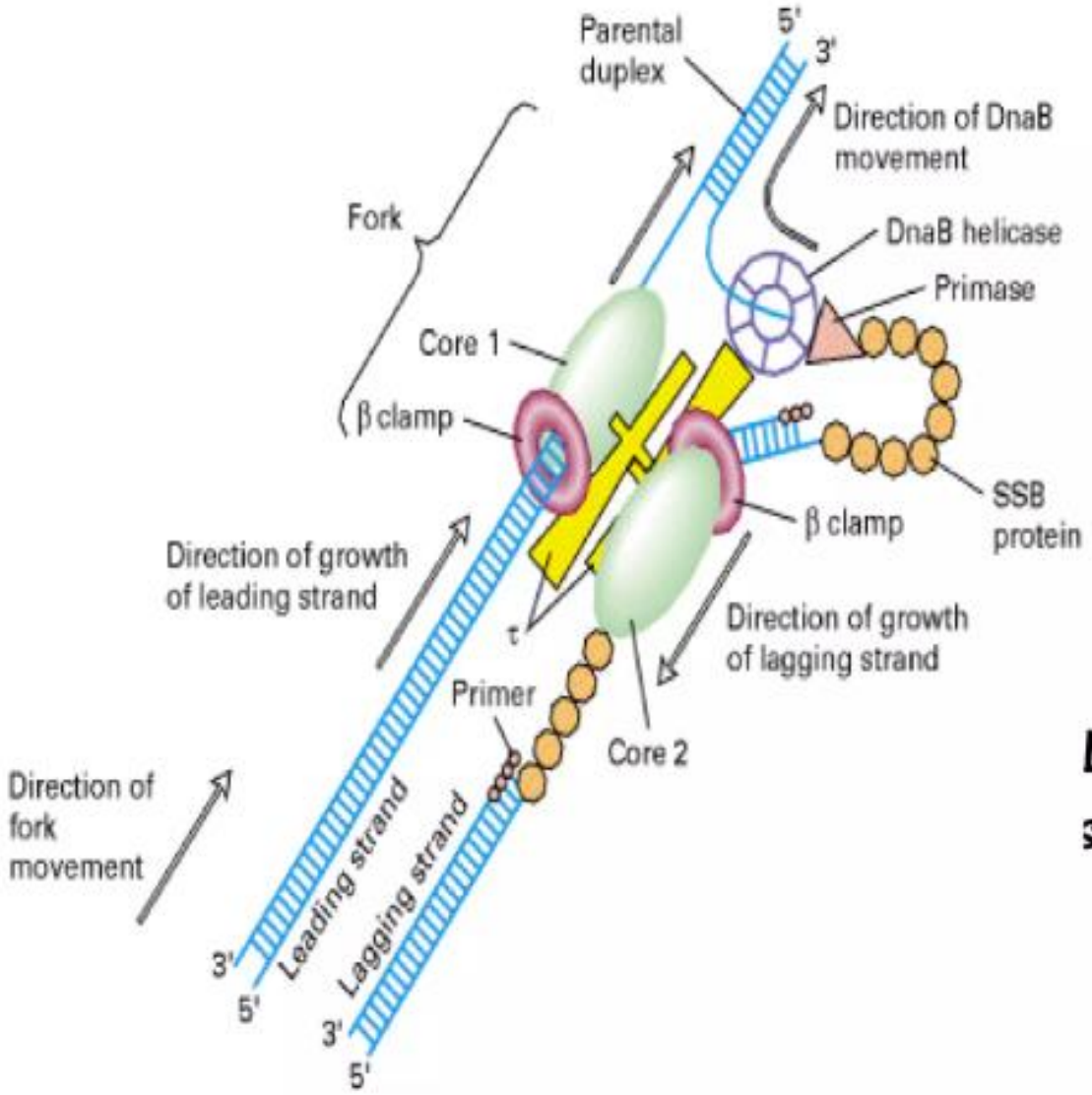
from Lodish *et al.*, Molecular Cell Biology, 4th ed. Fig 12-8

DnaB unwinds DNA in the 5'-3' direction

DnaB uses ATP hydrolysis to separate the strands

SSB proteins prevent the separated strands from re-annealing

Coordination of Leading and Lagging Strand Synthesis



Two molecules of Pol III are bound at each growing fork and are held together by τ

The size of the DNA loop increases as lagging strand is synthesized

Lagging strand polymerase is displaced when Okazaki fragment is completed and rebinds to synthesize the next Okazaki fragment

from Lodish *et al.*, Molecular Cell Biology, 4th ed. Fig 12-11

Direction of Replication:

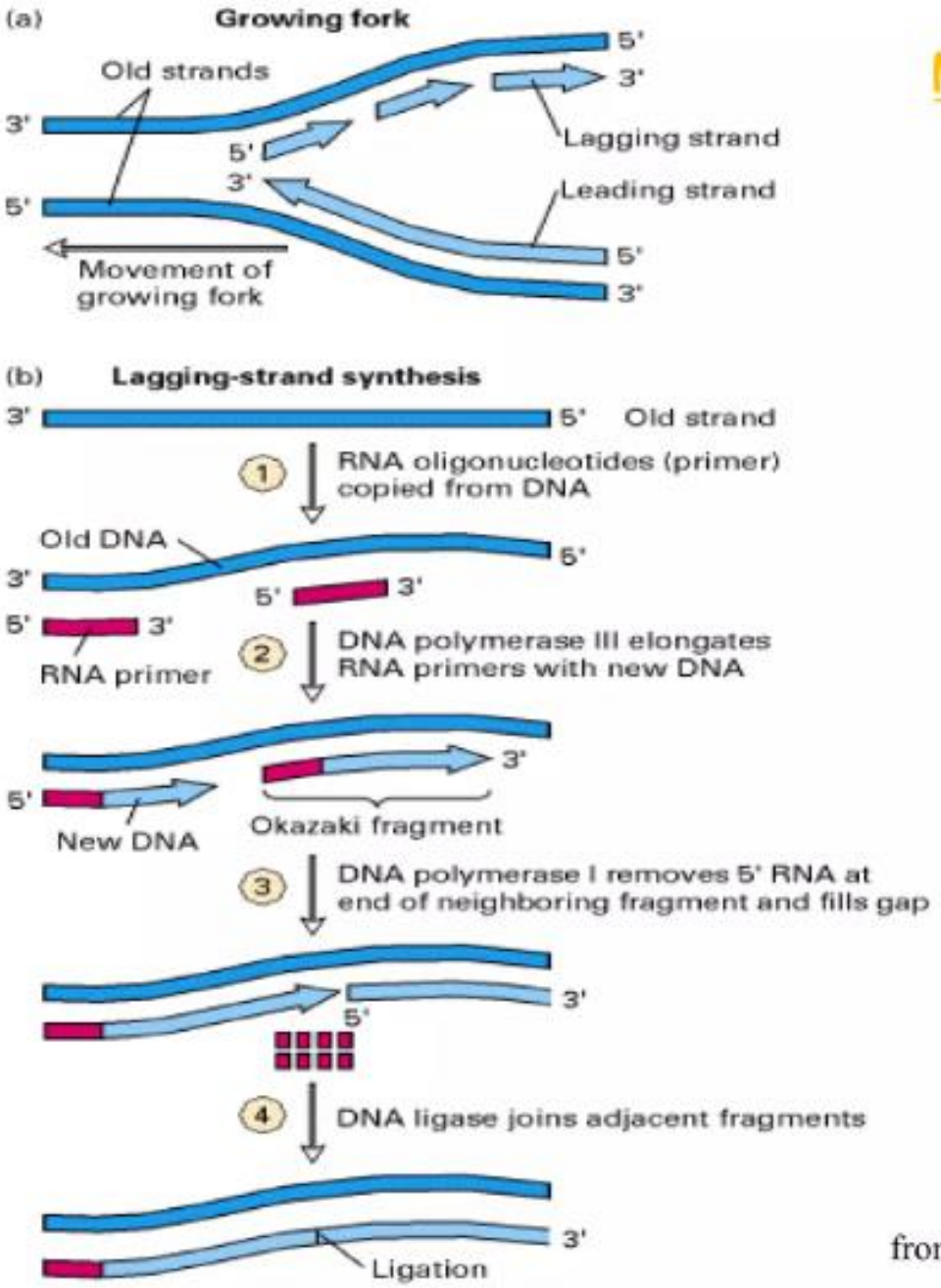
- Once initiated, DNA replication assembly proceeds along the DNA molecule, and the precise point at which replication is occurring is termed as the replication fork.

- Generally, in both prokaryotes and eukaryotes, the process of DNA replication proceeds in two opposite directions, from the origin of replication.

- However, in certain plasmids present in bacterial cells, unidirectional DNA replication has been observed.

- These plasmids replicate through the rolling circle model, wherein multiple linear copies of the circular DNA are synthesized and then circularized.

Mechanics of DNA Replication in *E. coli*



Leading strand is synthesized continuously and lagging strand is synthesized as Okazaki fragments

The 5' to 3' exonuclease activity of Pol I removes the RNA primer and fills in the gap

DNA ligase joins adjacent completed fragments

from Lodish *et al.*, Molecular Cell Biology, 4th ed. Fig 12-9

Enzymes: Although a similar set of enzymes are involved in prokaryotic and eukaryotic DNA replication, the latter one is more complex and varied. The initiator proteins, single-stranded DNA-binding protein (SSB), primase, DNA helicase, and DNA ligase are present in both prokaryotes and eukaryotes.

Enzymes specific to prokaryotes:

Enzyme	Activity
DNA Polymerase I	5' to 3' polymerase, 3' to 5' exonuclease, 5' to 3' exonuclease
DNA Polymerase III	5' to 3' polymerase, 3' to 5' exonuclease

Enzymes specific to eukaryotes:

Enzyme	Activity
DNA polymerase α	5' to 3' polymerase
DNA polymerase δ	5' to 3' polymerase, 3' to 5' exonuclease
DNA polymerase ε	5' to 3' polymerase

- In addition, eukaryotes contain DNA polymerase γ, which is involved in mitochondrial DNA replication.

- Also, the topoisomerases, enzymes that regulate the winding and unwinding of DNA during the movement of replication fork, differ in their activity.

- Prokaryotes, generally use type II topoisomerase called DNA gyrase, that introduces a nick in both the DNA strands.

- On the contrary, most eukaryotes utilize type I topoisomerases, that cut a single strand of DNA, during the movement of the replication fork.

Okazaki fragments:

- During DNA replication, the synthesis of one strand occurs in a continuous manner, whereas that of the other strand occurs in a discontinuous manner through the formation of fragments. The former strand is termed as the leading strand, the latter as the lagging strand, and the intermediate fragments are termed as the Okazaki fragments. The reason for such a difference is the antiparallel nature of DNA strands, as against the unidirectional activity of the DNA polymerase.

- Prokaryotic Okazaki fragments are longer, with the typical length observed in *Escherichia coli (E. coli)* being about 1000 to 2000 nucleotides.

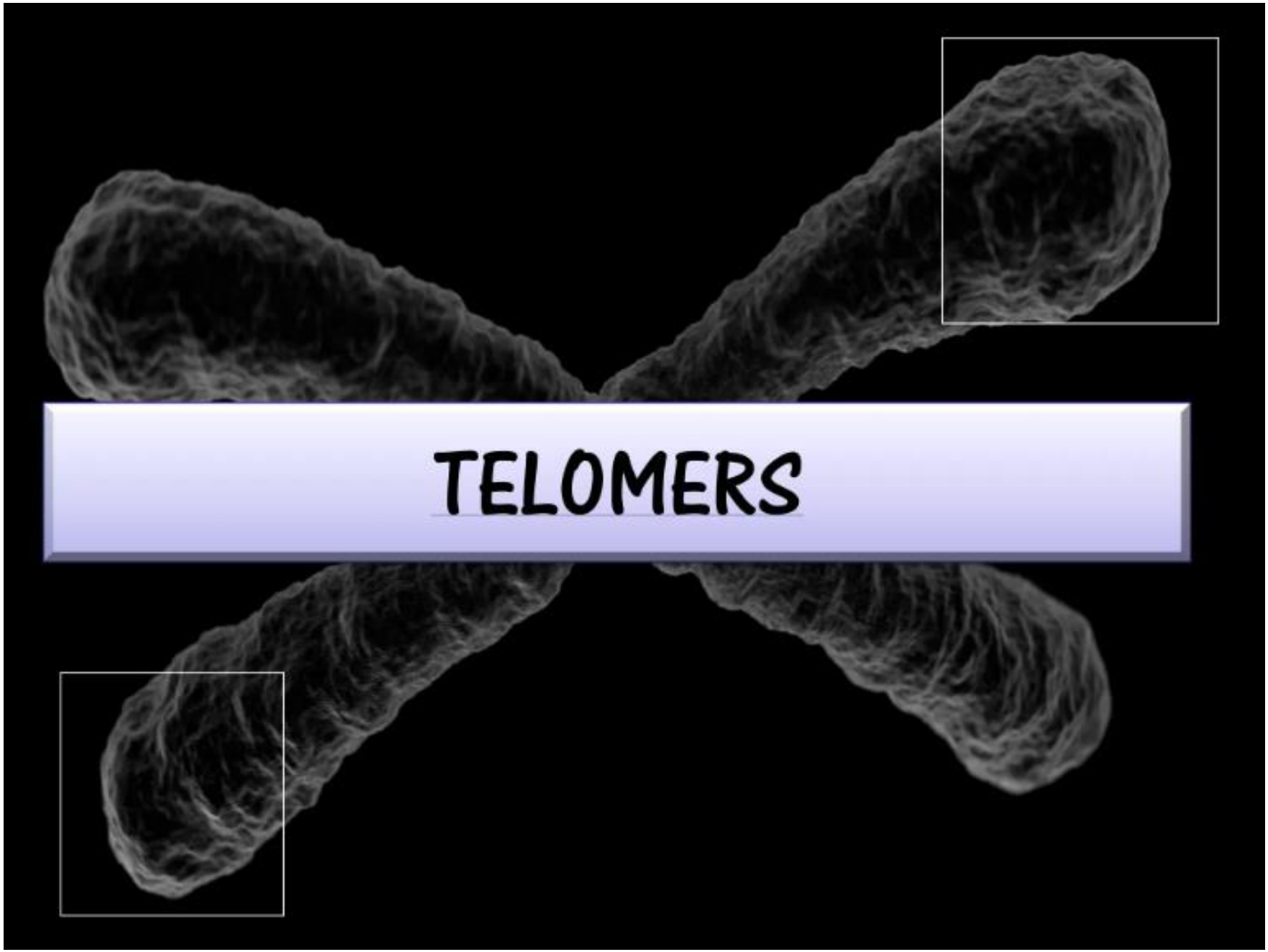
- The length of eukaryotic Okazaki fragments ranges between 100 and 200 nucleotides. Although comparatively shorter, they are produced at a rate slower than that observed in prokaryotes.

Termination:

- The termination of DNA replication occurs at specific termination sites in both prokaryotes and eukaryotes.

- In prokaryotes, a single termination site is present midway between the circular chromosome. The two replication forks meet at this site, thus, halting the replication process.

- In eukaryotes, the linear DNA molecules have several termination sites along the chromosome, corresponding to each origin of replication. However, the eukaryotic DNA replication is characterized by a unique end-replication problem, wherein a part of DNA present at the ends of the chromosome does not get replicated. So, the lagging strand is shorter than the leading strand. This problem is addressed in eukaryotes by the presence of non-coding, repetitive DNA sequence called telomeres, at the ends of chromosomes.


TELOMERS

Telomeres are Specialized Structures at the Ends of Chromosomes:

- Telomeres contain multiple copies of short repeated sequences and contain a 3' -G-rich overhang. Forms a T-loop with the help of specialized proteins to protect itself from exonuclease activity.

- For example, Tetrahymena contains tandem TTGGGG repeats, whereas mammals have TTAGGG repeats. The telomerase mechanism has been best studied in Tetrahymena, where enzyme activity was first identified.

- Telomeres are bound by proteins which protect the telomeric ends initiate heterochromatin formation and facilitate progression of the replication fork.

- Telomeres protect chromosome ends from being processed as a ds break. End-protection relies on telomere-specific DNA conformation, chromatin organization and DNA binding proteins

- For her discovery of telomerase and its action, Elizabeth Blackburn received the Nobel Prize for Medicine and Physiology in 2009.

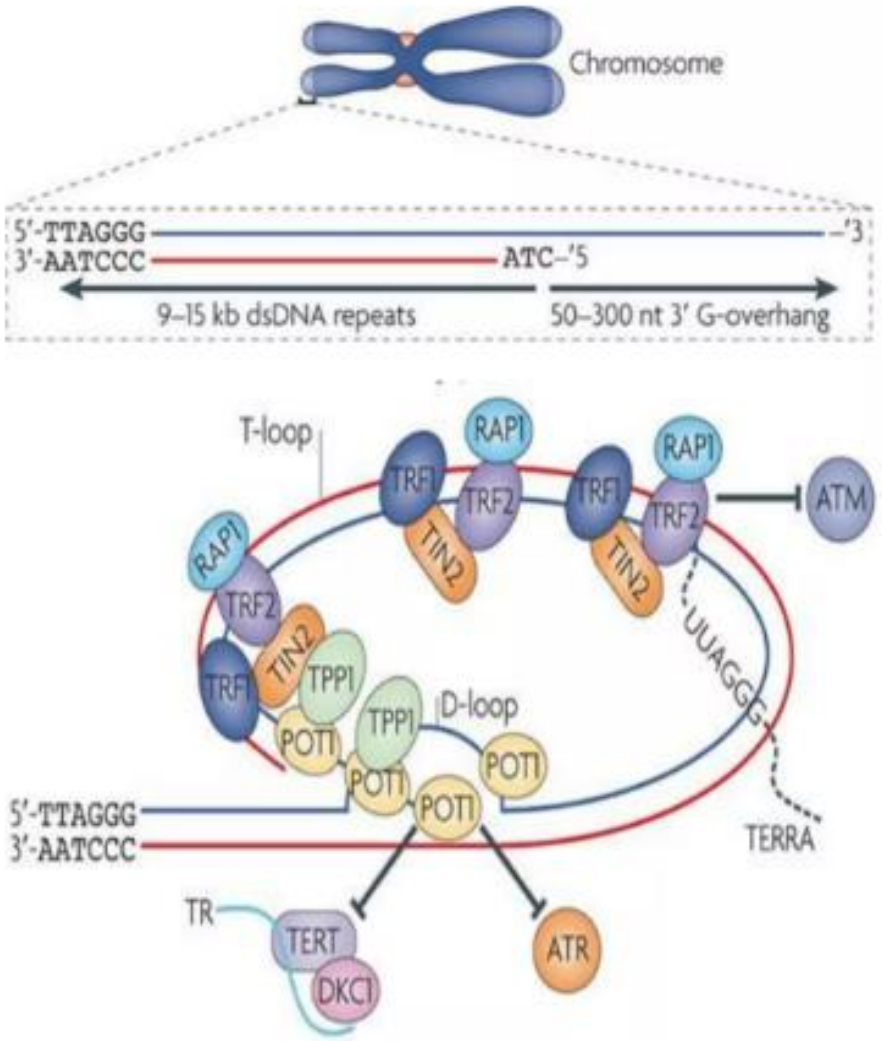
- In humans, a six base pair sequence, TTAGGG, is repeated 100 to 1000 times.

The discovery of the enzyme telomerase helped in the understanding of how chromosome ends are maintained. The telomerase enzyme contains a catalytic part and a built-in RNA template. It attaches to the end of the chromosome, and complementary bases to the RNA template are added on the 3' end of the DNA strand. Once the 3' end of the lagging strand template is sufficiently elongated, DNA polymerase can add the nucleotides complementary to the ends of the chromosomes. Thus, the ends of the chromosomes are replicated.

Organism	Telomeric DNA sequence	RNA template sequence
H. sapien	5'-T_2AG_3	3'-UCCCAAUC
S. cerevisiae	5'-$T_{1\text{-}6}GTG_{2\text{-}3}$	3'-CACACACCCACACCAC
A. thaliana	5'-T_3AG_3	unidentified

- Telomere length serves as intrinsic biological clock at regulating life span of the cell.
- Hayflick limit maximal number of cell division that a cell can achieve in vitro.
- When cells reach this limit they undergo morphological and biochemical changes that eventually lead to arrest of cell proliferation a processes called cell senescence.

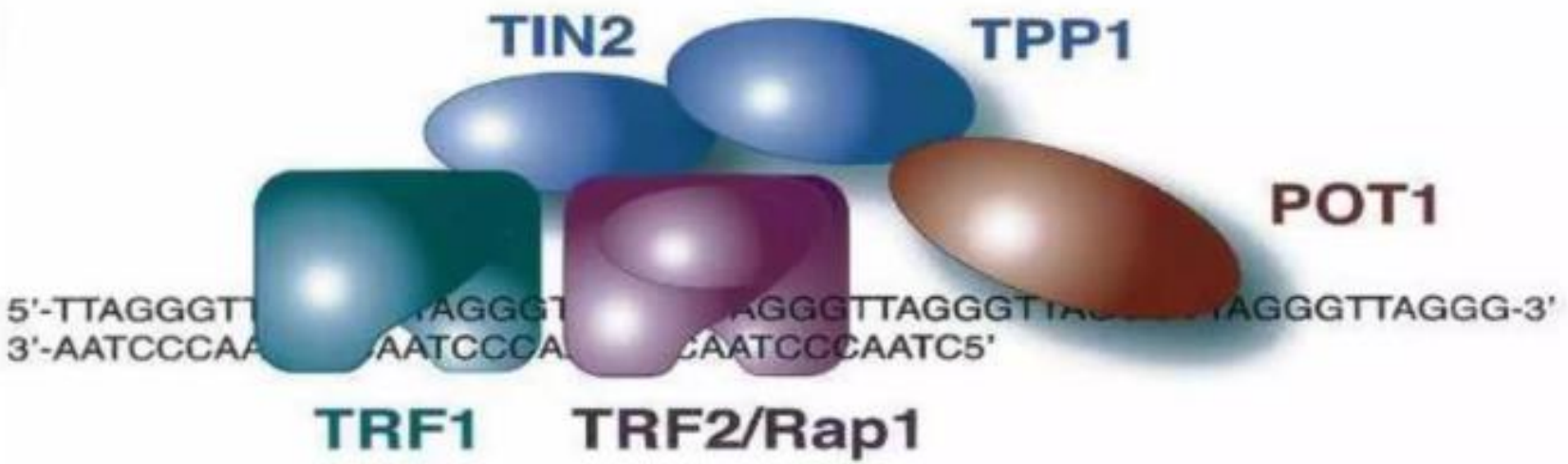
Structure of Human Telomeres



from O' Sullivan and Karlseder, *Nature Rev.Mol.Cell Biol.* 11, 171 (2010)

- ✓ Telomeres consist of numerous short dsDNA repeats and a 3'-ssDNA overhang.
- ✓ The G-tail is sequestered in the T-loop.
- ✓ Shelterin is a protein complex that binds to telomeres.
- ✓ TRF2 inhibits ATM-dependent DNA damage response.
- ✓ Shelterin components block telomerase activity.

Shelterin Specifically Associates with Telomeres

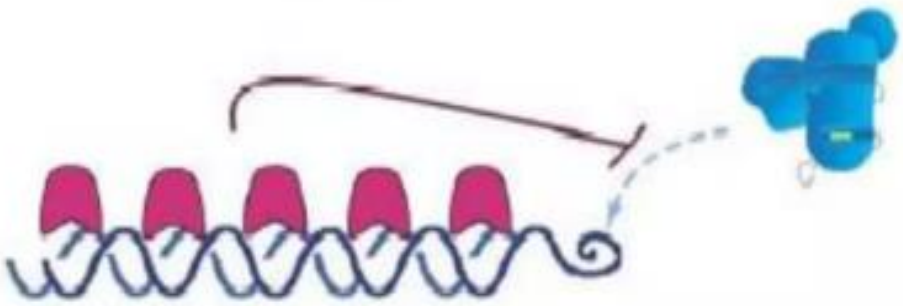


from de Lange, *Genes Dev.* **19**, 2100 (2005)

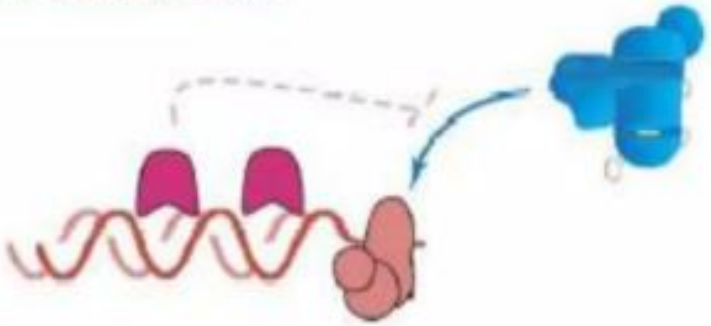
- ✓ Shelterin subunits specifically recognize telomeric repeats.
- ✓ Shelterin allows cells to distinguish telomeres from sites of DNA damage.

Telomerase Action is Restricted to a Subset of Ends

Telomerase non-extendible state



Telomerase extendible state



from Bertuch and Lundblad, *Curr:Opin.Cell Biol.* **18**, 247 (2006)

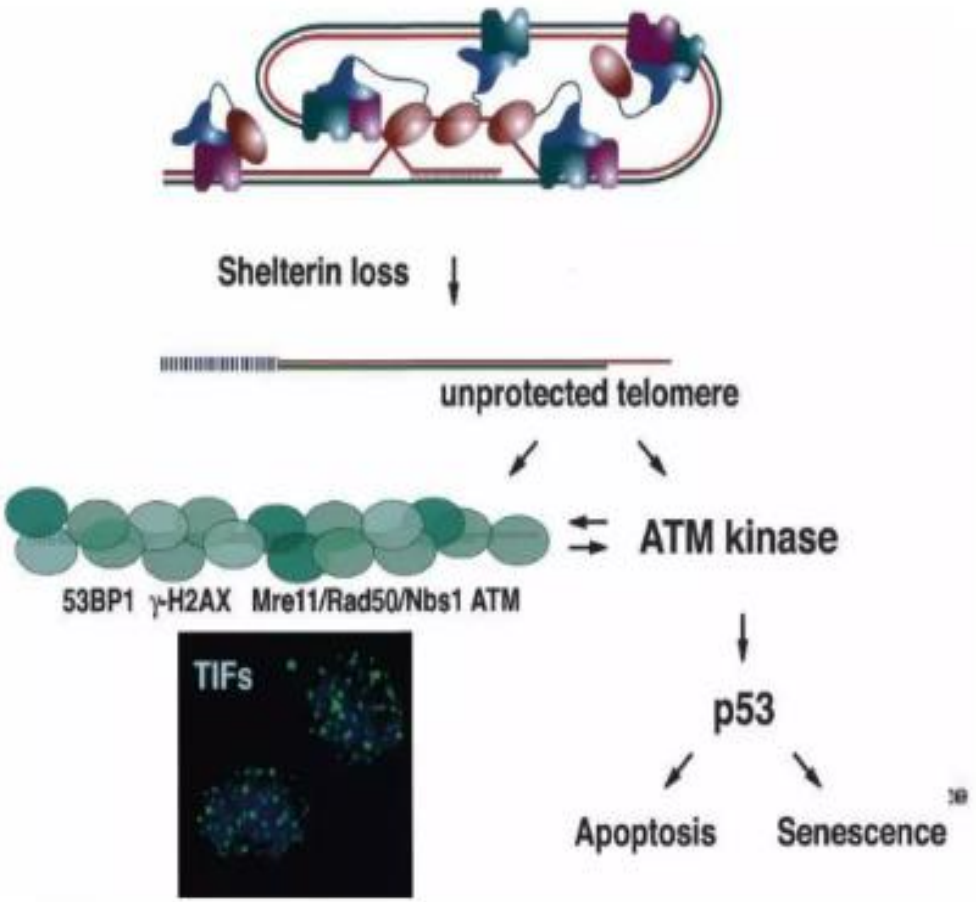
Telomere length is regulated by shelterin.

Increased levels of shelterin inhibits telomerase action.

Telomerase is inhibited by increased amounts of POT1 (protection of telomeres 1)

Elongation of shortened telomeres depends on the recruitment of the Est1 subunit of telomerase by Cdc13 end-binding protein

Dysfunctional Telomeres Induce the DNA Damage Response



from de Lange, *Genes Dev.* **19**, 2100 (2005)

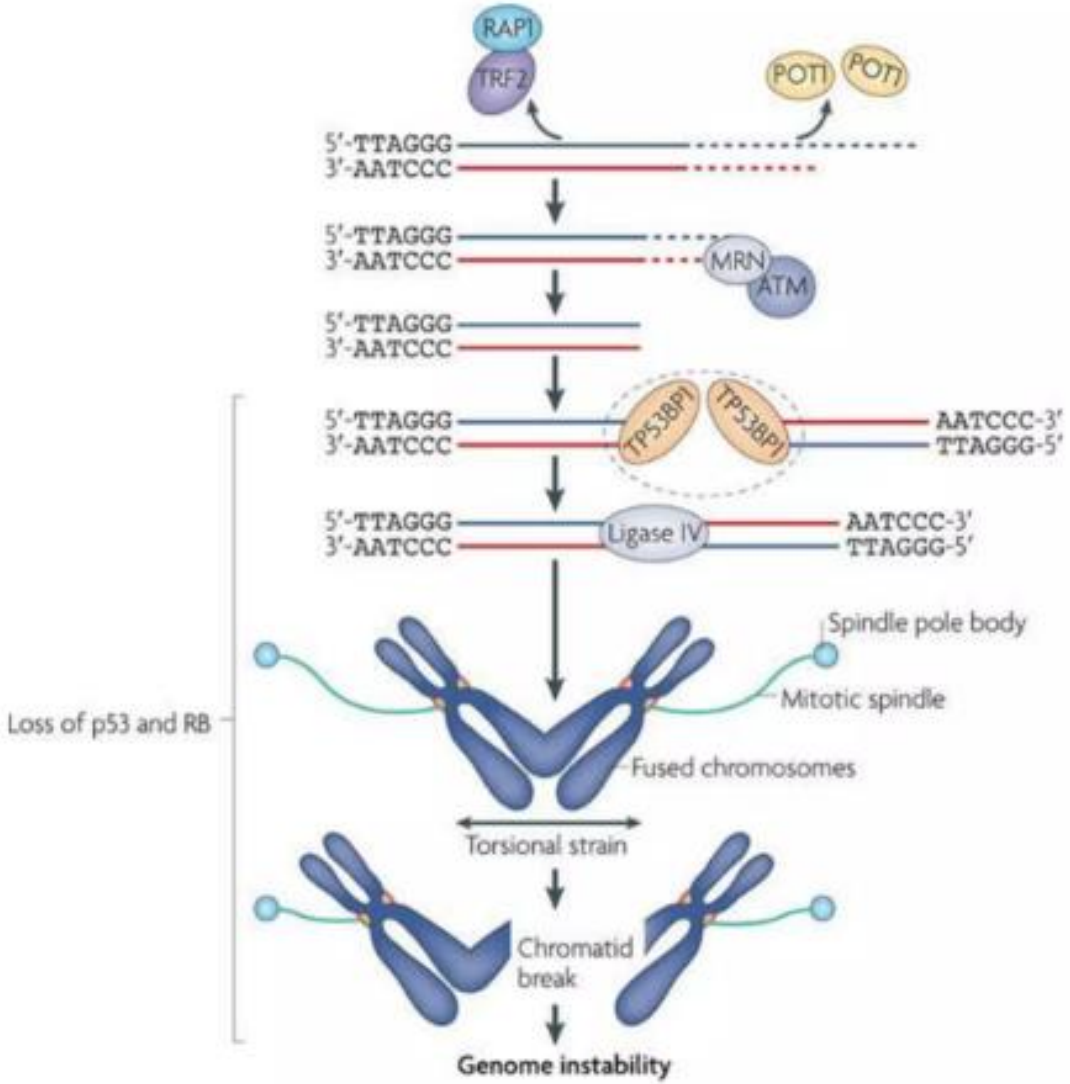
Shelterin may contain an ATM inhibitor.

Telomere damage activates ATM (ataxia telangiectasia mutated).

DNA damage response proteins accumulate at unprotected telomeres.

ATM activates p53 and leads to cell cycle arrest or apoptosis.

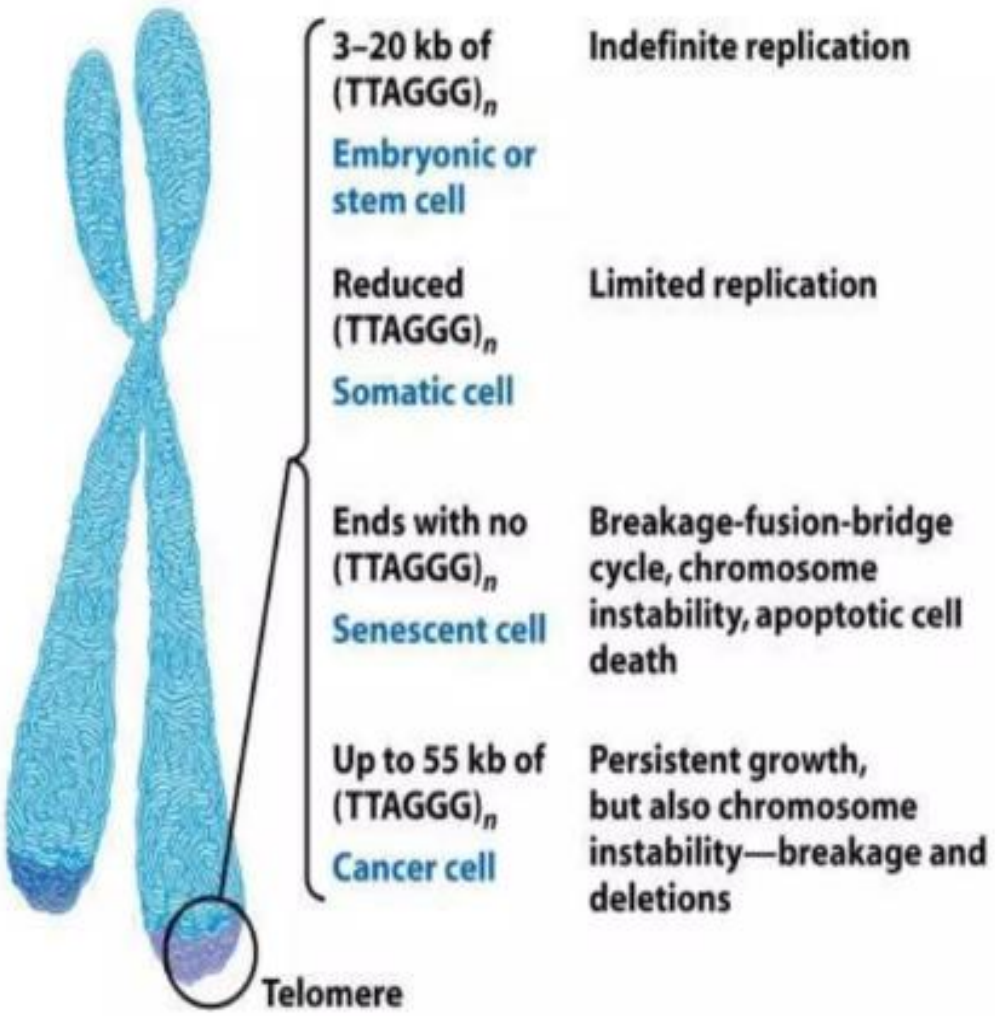
Loss of Functional Telomeres Results in Genetic Instability



- ✓ Dysfunctional telomeres activate DSB (DNA double-strand breaks) repair by NHEJ (non-homologous end-joining).
- ✓ Fused chromosomes result in chromatid break and genome instability

from O' Sullivan and Karlseder, *Nature Rev.Mol.Cell Biol.* 11, 171 (2010)

Loss of Telomeres Limits the Number of Rounds of Cell Division



from Lodish *et al.*, Molecular Cell Biology, 6th ed. Fig 25-31

- Stem cells and germ cells contain telomerase which maintains telomere size.
- Somatic cells have low levels of telomerase and have shorter telomeres.
- Loss of telomeres triggers chromosome instability or apoptosis.
- Cancer cells contain telomerase and have longer telomeres.

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 the case?

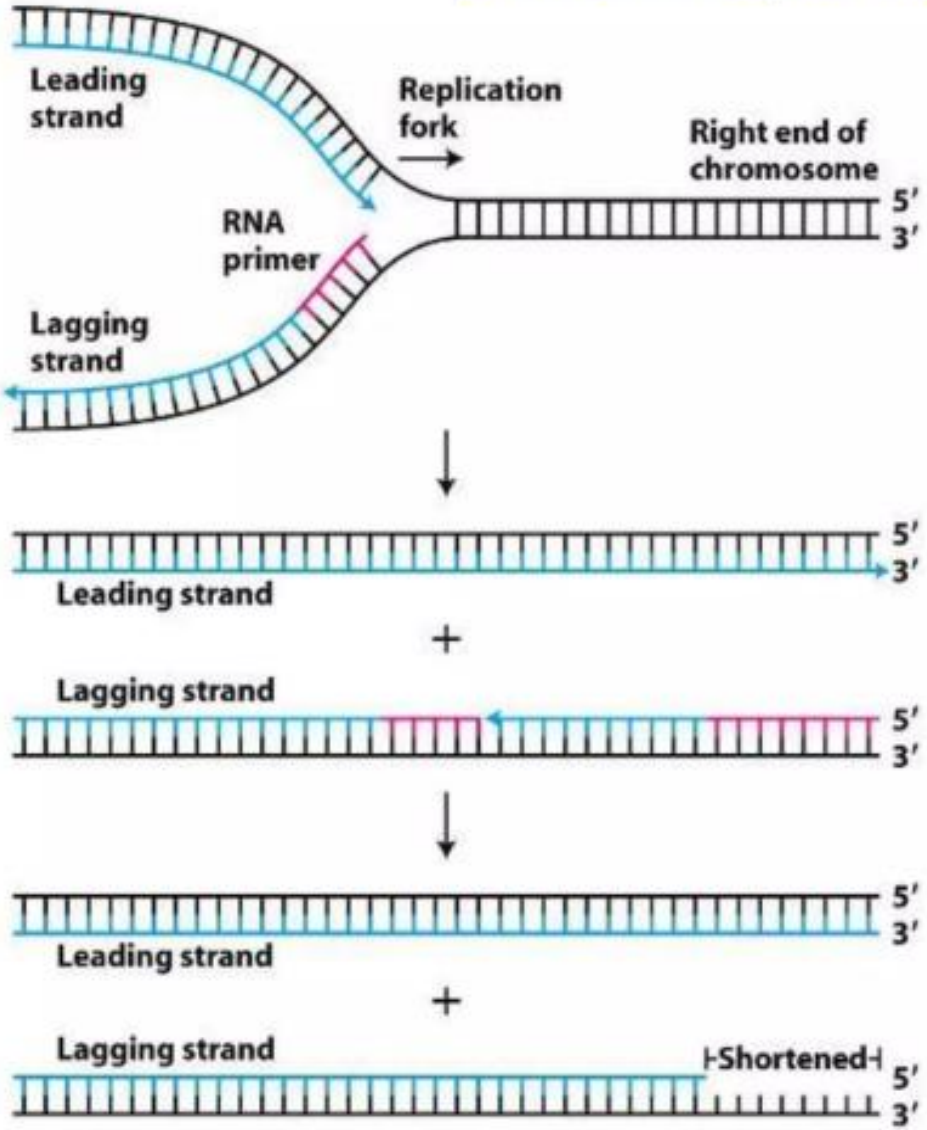
- When DNA is being copied, one of the two new strands of DNA at a replication fork is made continuously and is called the leading strand.
- The other strand is produced in many small pieces called Okazaki fragments, each of which begins with its own RNA primer, and is known as the lagging strand.

- 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^llstart superscript, l, end superscript.

- Also, the primer of the last Okazaki fragment that *does* get made can't be replaced with DNA like other primers.

The End Replication Problem



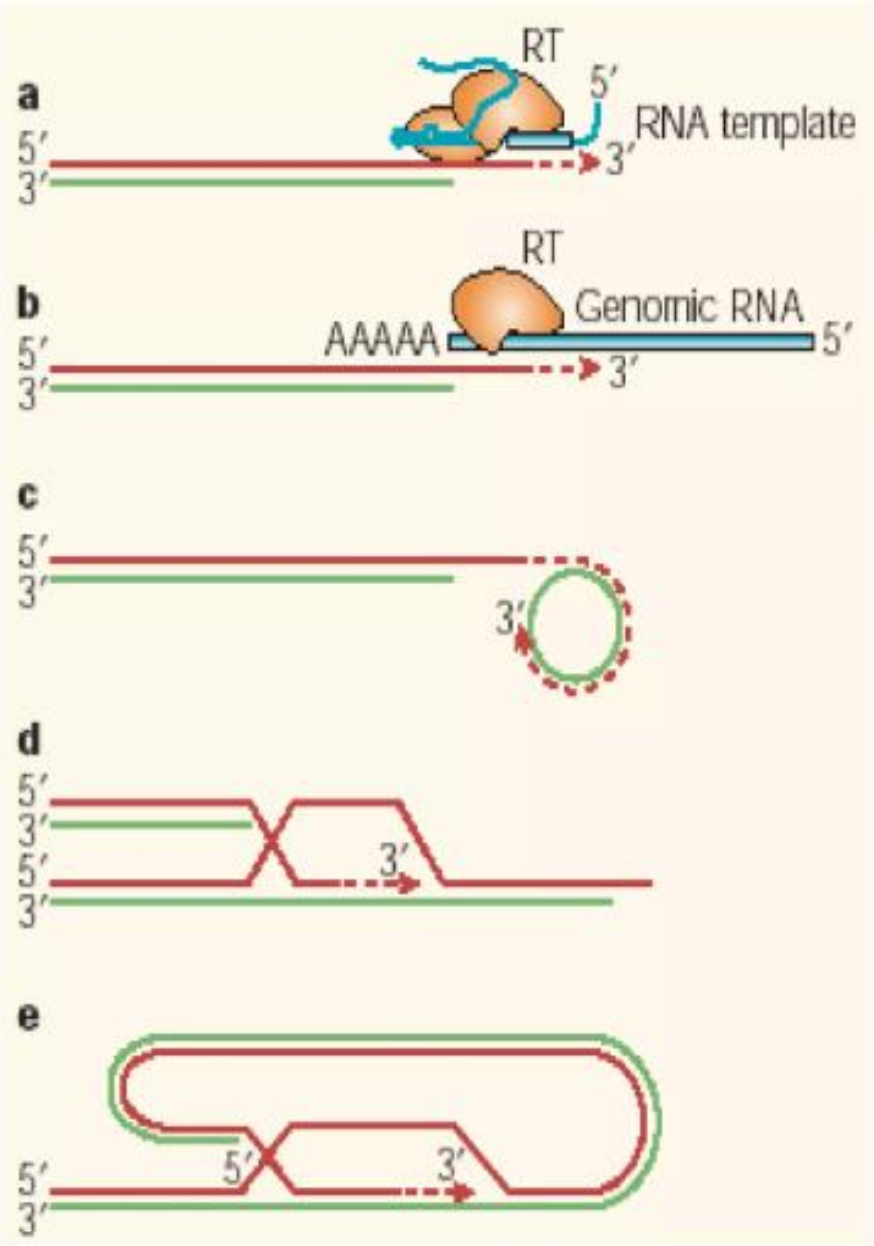
from Lodish *et al.*, Molecular Cell Biology, 6th ed. Fig 6-49

- Leading strand is synthesized to the end of the chromosome.
- Lagging strand utilizes RNA primers which are removed.
- The lagging strand is shortened at each cell division

➢ Why is that the case?

➢ 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.

Solutions to the End Replication Problem



- 3' -terminus is extended using the reverse transcriptase activity of telomerase
- Dipteran insects use retrotransposition with the 3'-end of the chromosome as a primer
- *Kluyveromyces lactis* uses a rolling circle mechanism in which the 3' -end is extended on an extrachromosomal template
- Telomerase-deficient yeast use a recombination- dependent replication pathway in which one telomere uses another telomere as a template.
- Formation of T-loops using terminal repeats allow extension of invaded 3'-ends

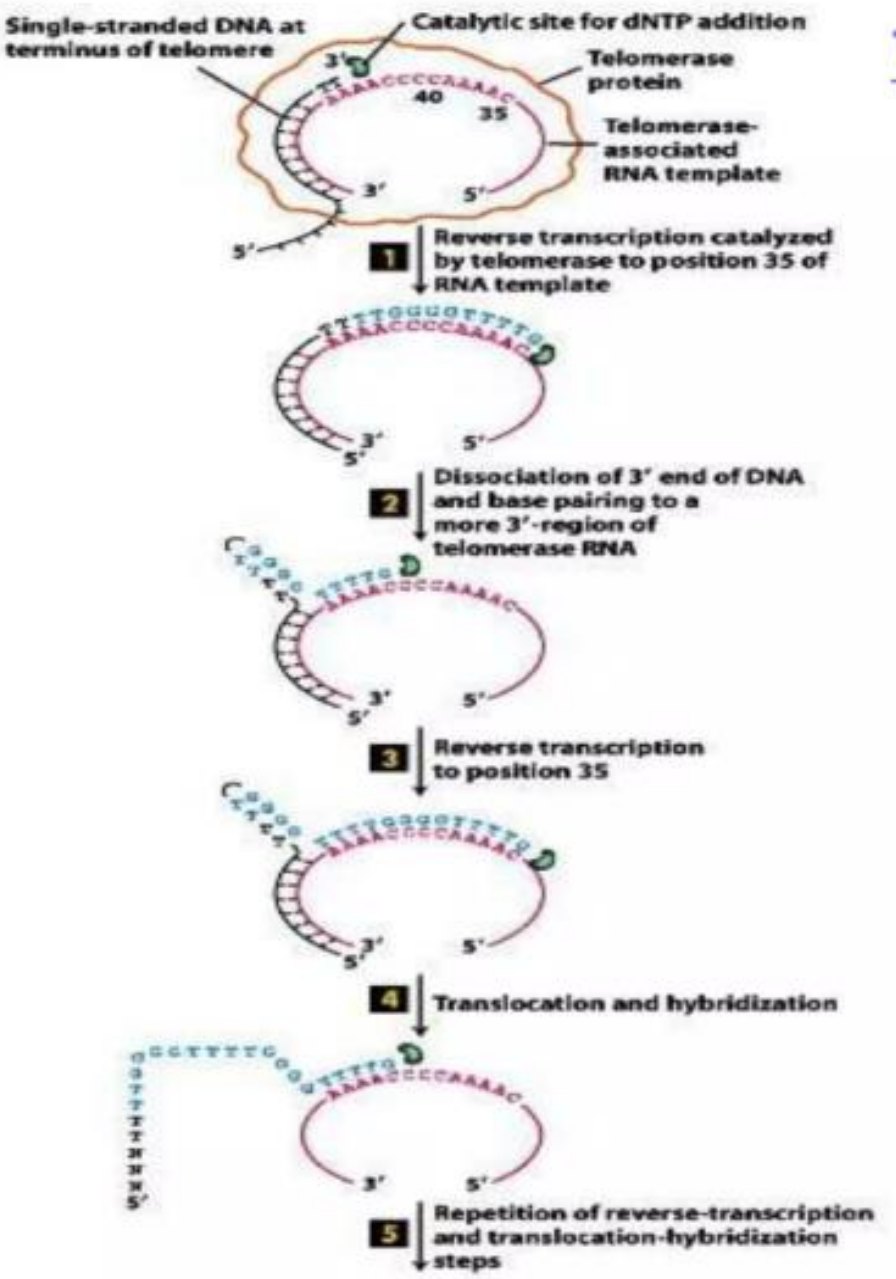
What is telomerase?

Telomerase is an enzyme with an inbuilt RNA template, that can replicate telomere sequences and keep the telomeres from getting shorter. DNA polymerase can then extend the DNA using the primer. In this way, the ends of the chromosomes are protected. Telomerase is active only in embryonic cells and in cells involved in the production of gametes (eggs and sperm).

How does telomerase work?

Telomerase is an enzyme that has a protein part and an RNA part. The RNA part of telomerase has a nucleotide sequence that can base-pair with the sequences in the telomere. When the RNA base-pairs with the sequence at the ends of the chromosome, it provides a template for the synthesis of new telomere repeats.

Notice that, in contrast to the usual situation where DNA serves as a template for the synthesis of RNA, in this situation, the RNA is acting as the template for the synthesis of DNA.



from Lodish *et al.*, Molecular Cell Biology, 6th ed. Fig 6-49

Telomerase Extends the ss 3' -Terminus

- Telomerase-associated RNA base pairs to 3' -end of lagging strand template
- Telomerase catalyzes reverse transcription to a specific site
- 3' -end of DNA dissociates and base pairs to a more 3' -region of telomerase RNA
- Successive reverse transcription, dissociation, and re-annealing extends the 3' -end of lagging strand template.
- New Okazaki fragments are synthesized using the extended template.

➢ How does telomerase make new telomere repeats?

- The protein component of telomerase is a *reverse transcriptase*, an enzyme that can make DNA copies from RNA templates.
- Telomerase can copy the sequence of the RNA bound to the end of the chromosome to make DNA copies of the telomere sequence. As a result of the action of telomerase, telomere sequences are added onto the end of the chromosome, preventing shortening of the chromosomes.

➢ Telomerase-based Cancer Therapy

Telomerase is widely expressed in cancers. 80-90% of tumors are telomerase-positive.

✓ Strategies include:

1) Direct telomerase inhibition
2) Telomerase immunotherapy

- **Telomerase and Aging:** Telomerase is typically active in germ cells and adult stem cells. It 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.