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FACULTY OF ENGINEERING &TECHNOLOGY

DEPARTMENT OF BIOTECHNOLOGY

- In **Eukaryotic DNA Replication** occurs in the **S phase of the cell cycle.**
- Eukaryotic DNA Replication is bidirectional occurring at the multiple sites simultaneously .
- The **Replication origins** are present in **clusters** called **Replication units.** In human ,there are about 100 ori of replication consisting of 1000 base pairs each.
- Each **replicon** consist of replication bubbles with two replication forks moving in opposite directions. **Replication** continues until the replication bubbles merge together.
- **The mechanism is similar to that seen in prokaryotes.**
- There are 5 different types of DNA polymerases which catalyze replication and repair . **(Pol α, Pol β, Pol γ, Pol ε, Pol δ)**

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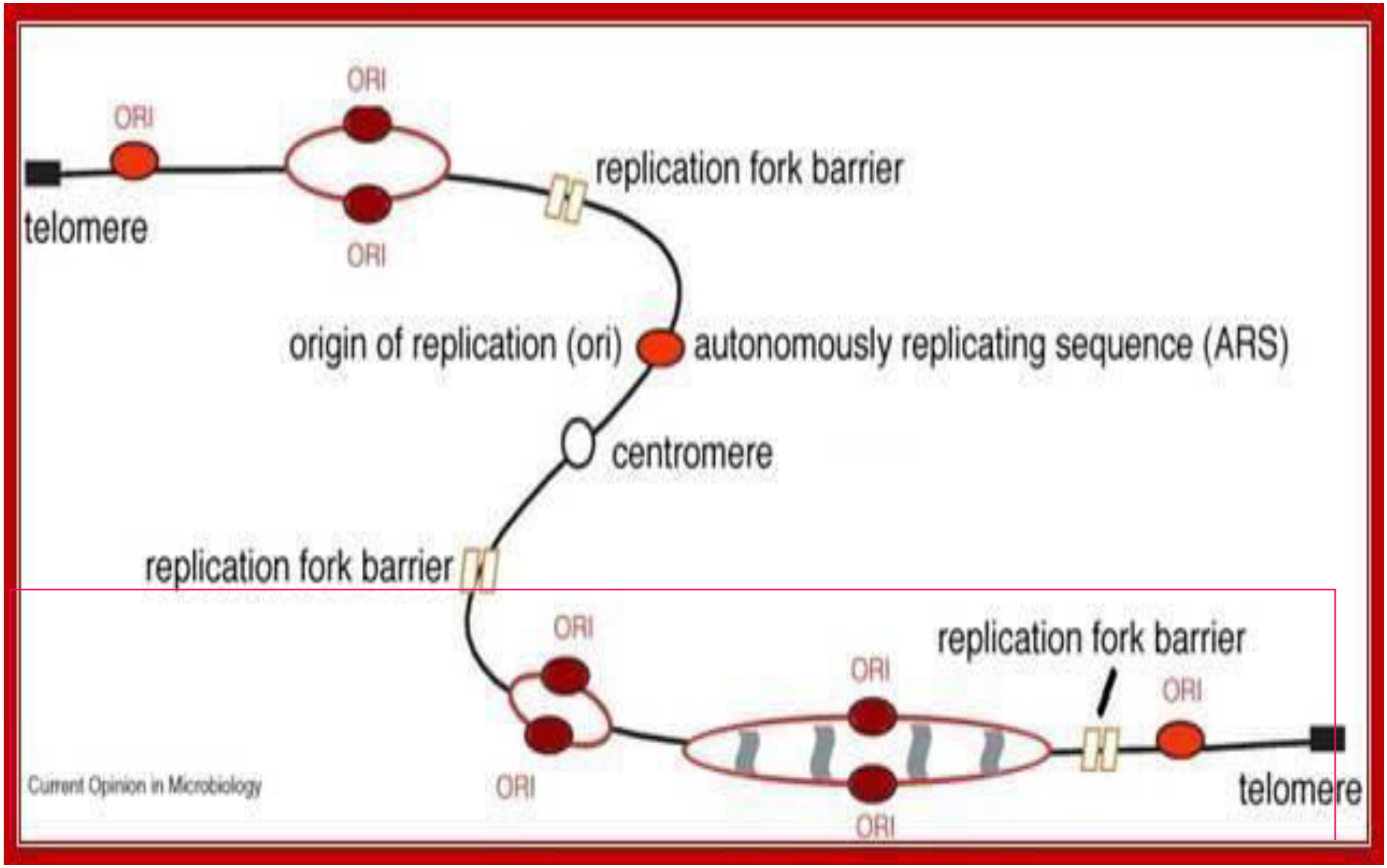
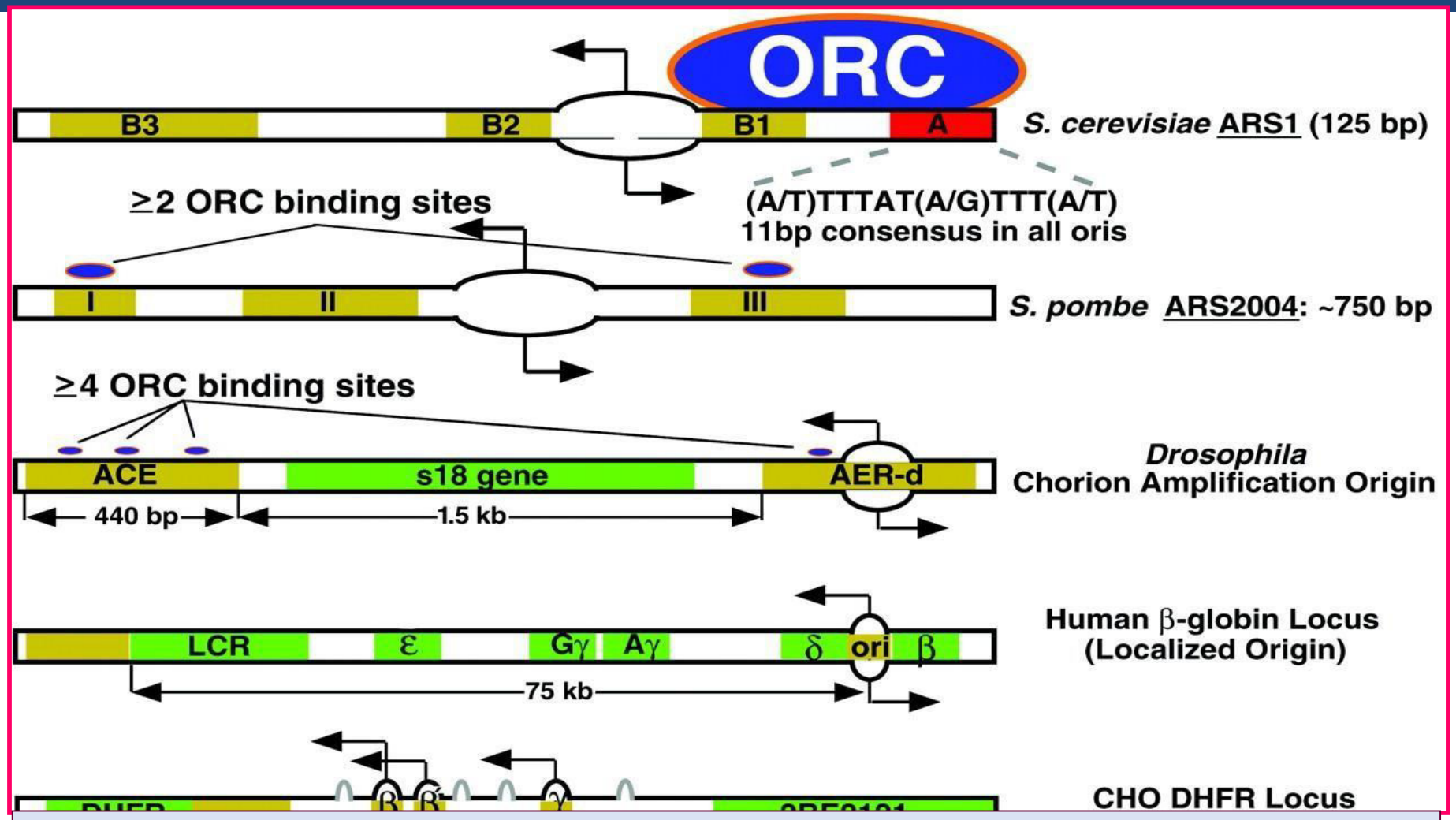

ORI
ORI
ORI
telomere
replication fork barrier
origin of replication (ori)
autonomously replicating sequence (ARS)
centromere
replication fork barrier
ORI
ORI
ORI
ORI
replication fork barrier
ORI
telomere
Current Opinion in Microbiology


ORC
B3
B2
B1
A
S. cerevisiae ARS1 (125 bp)
≥2 ORC binding sites
(A/T)TTTAT(A/G)TTT(A/T)
11bp consensus in all oris
I
II
III
S. pombe ARS2004: ~750 bp
≥4 ORC binding sites
ACE
s18 gene
AER-d
440 bp
1.5 kb
Drosophila
Chorion Amplification Origin
LCR
ε
Gγ
Aγ
δ
ori
β
75 kb
Human β-globin Locus
(Localized Origin)
CHO DHFR Locus

- **Functions of proliferating cell nuclear antigen (PCNA): PCNA** binds to DNA polymerase δ (function similar to polymerase III of E.Coli). The binding of PCNA to polymerase δ , increases enzyme processivity and starts replicating long stretches of deoxyribonucleotides .

- This process is called **polymerase switching** because polymerase δ replaces polymerase α.

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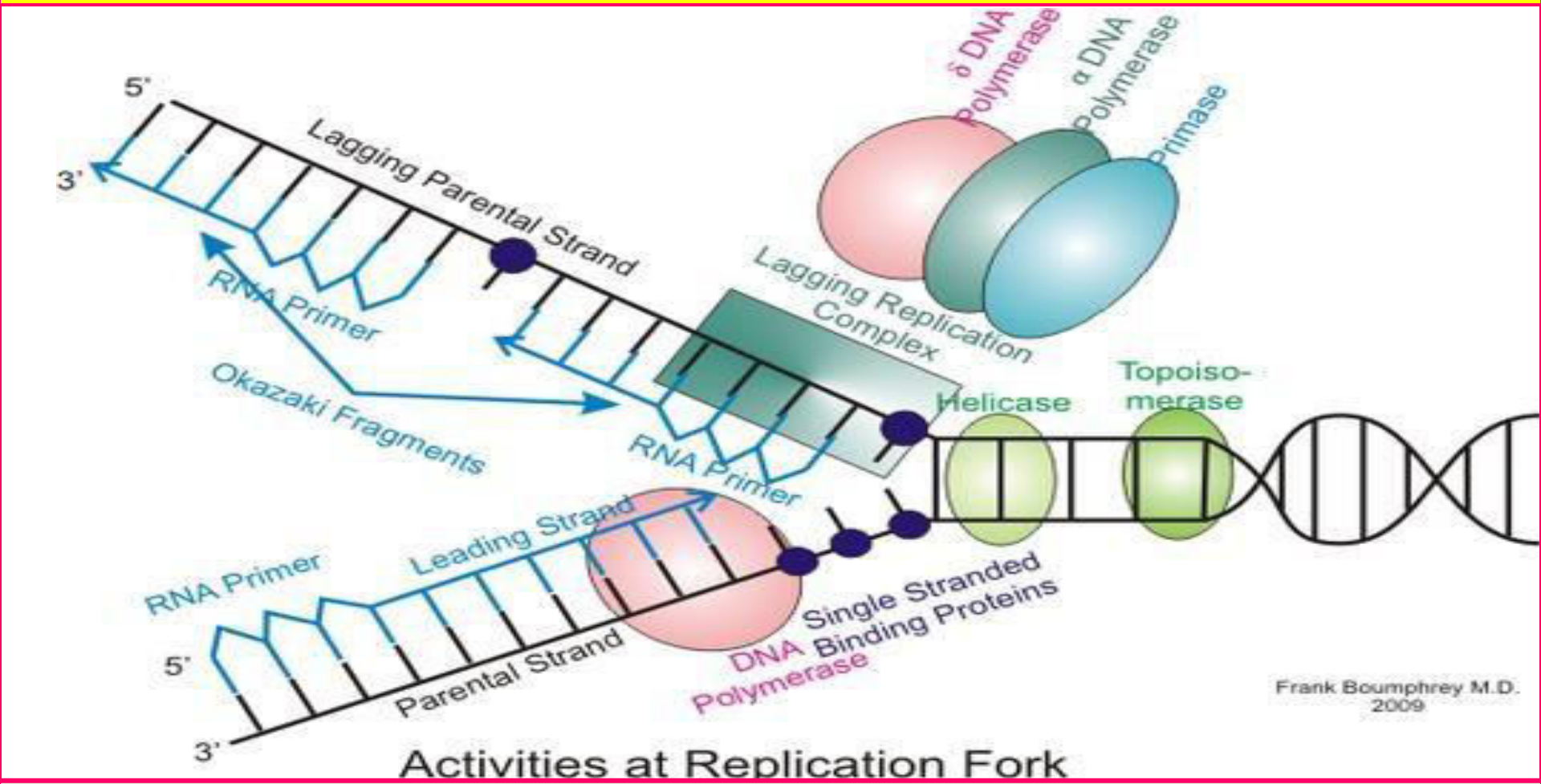
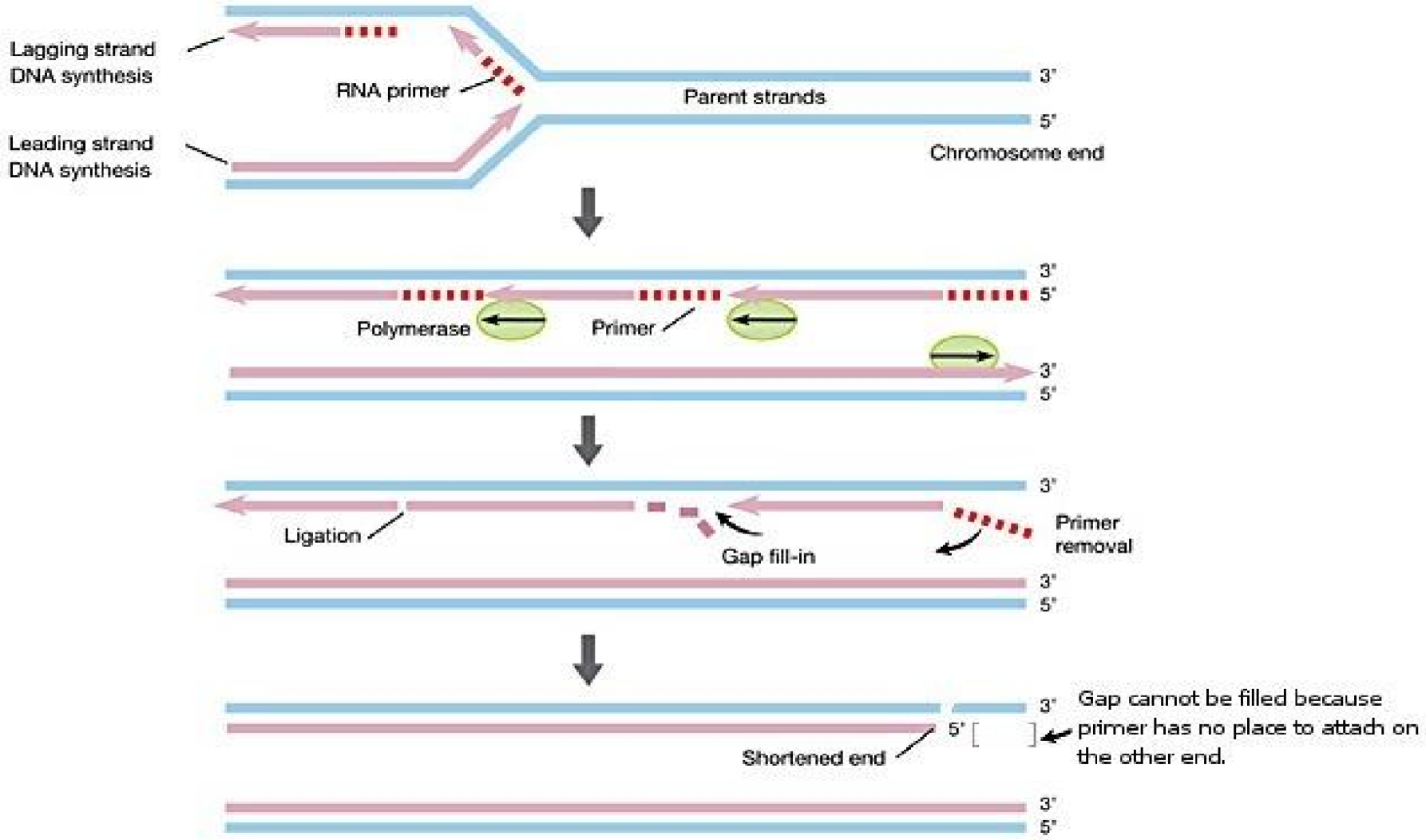
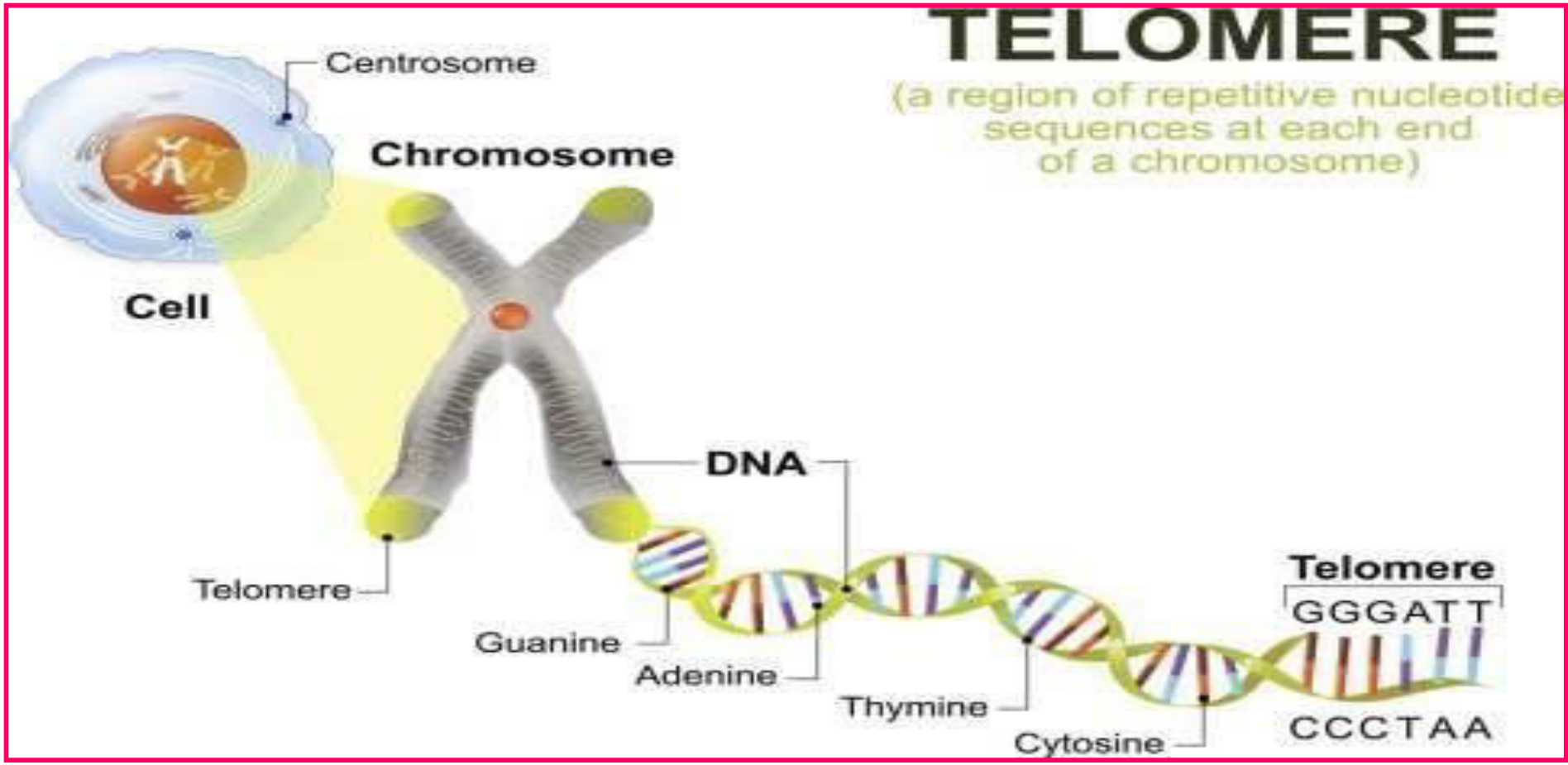


The mechanism of Eukaryotic DNA replication is similar to that seen in prokaryotes.


Lagging strand DNA synthesis
RNA primer
Parent strands
3'
5'
Leading strand DNA synthesis
Chromosome end
3'
5'
Polymerase
Primer
3'
5'
3'
Ligation
Gap fill-in
Primer removal
3'
5'
3'
5'
Gap cannot be filled because primer has no place to attach on the other end.
Shortened end
3'
5'



Replication always takes place from 5’to 3’ direction in the new strand. The DNA polymerase enzyme is not able to synthesize the new strand at the end of 5’ end of the new strand . In other words ,a small portion of (about 300 nucleotides couldn't be replicated).

Importance of Telomeres

- Replication always takes place from 5'to 3' direction in the new strand. The DNA polymerase enzyme is not able to synthesize the new strand at the end of 5' end of the new strand . In other words ,a small portion of (about 300 nucleotides couldn't be replicated).
- This end piece of chromosome is called as **Telomeres.** Therefore enzyme **Telomerase** or **Telomere Terminal transferase** takes up the job of replication of the end piece of chromosomes . The **Telomeres** are noncoding repetitive sequences .
- After the normal replication ,there is a single strand in this region, so the portion is degraded by exonucleases. This broken end leads to aberrant recombination or end to end fusions .
- Unless there is some mechanism to replicate telomeres ,the length of the chromosome will go on reducing at each cell division .The stability of the chromosomes is thus lost. Many genes might be lost in the process.
- The shortening of **Telomeres end** is prevented by an enzyme **Telomerase. It** contains **an RNA component ,**which provides the template for telomeric repeat synthesis.

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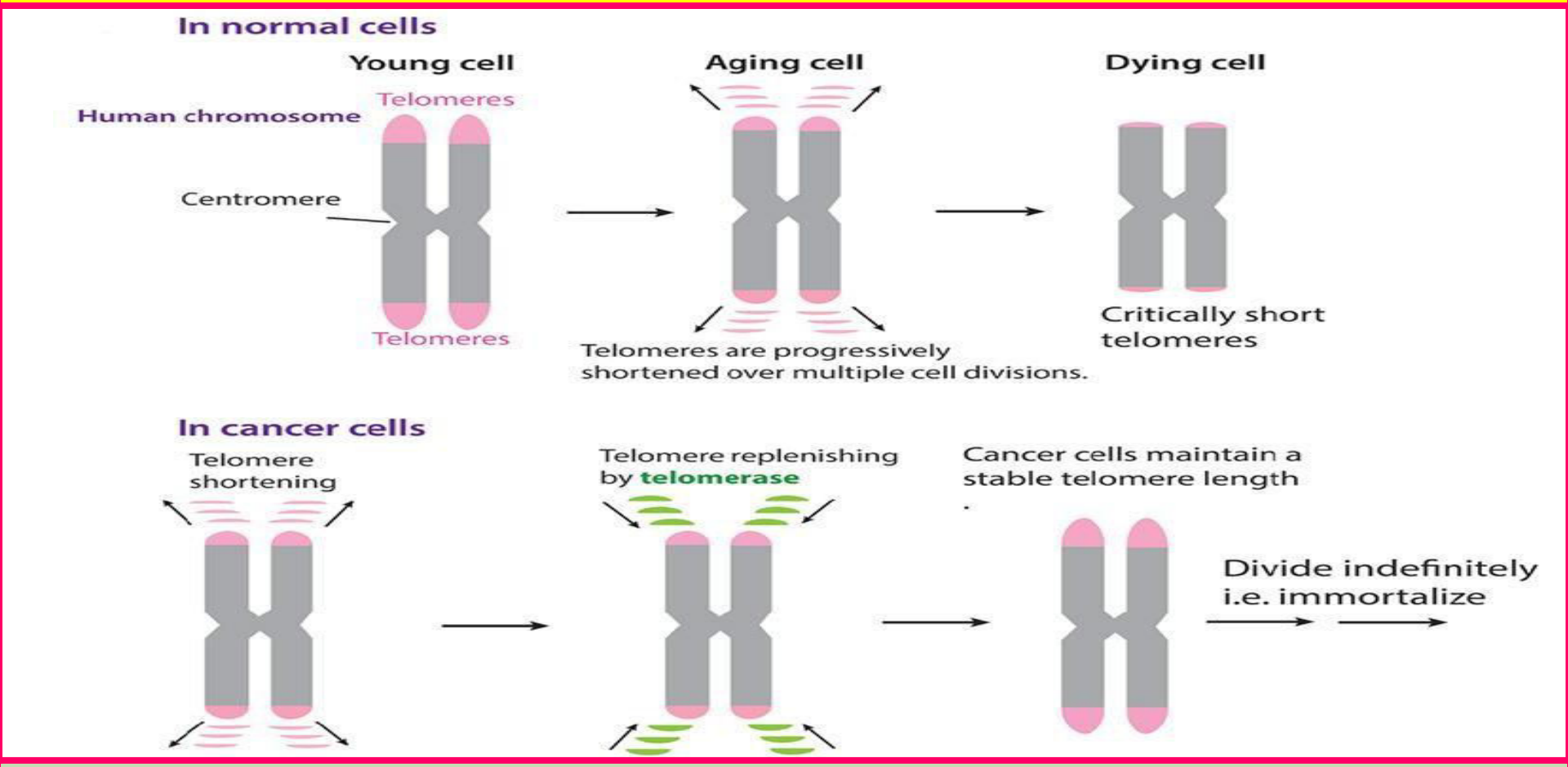
Telomeres and aging



Terminal restriction fragments from 70 years old individualsare shorter than those from 20years old individuals .Thus in old age ,the **Telomerase** activityis lost, leading to chromosome instability and cell death. Telomerase may be responsible for the immortalizationof cancer