Molecular Cell Biology A

"DNA Replication and Repair"

BIOX24ZL

Tuesdays 9-10:30

Ray LC

DNA replication is semiconservative: an elegant experiment.

- Complementary base pair allows replication with either strand by cluster of proteins
- Watson: DNA replication is semiconservative
- Delbruck: breaks and unions, patchwork of old and new nucleotides but no unwinding
- Others: conservative, daughter 2 new strands
- Meselson & Stahl: grow e. coli in N15 and N14 nitrogen media (heavy & light DNA), centrifuge in cesium chloride allows separation, grow N15 e.coli in N14 for one gen, heat up after to separate strands to differentiate with Delbruck

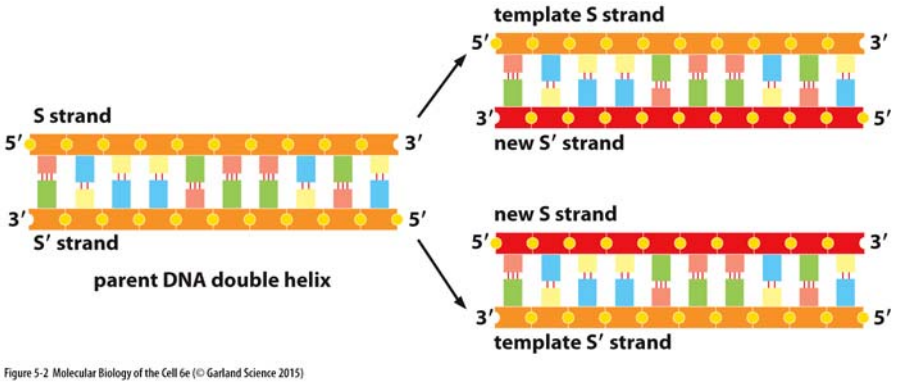


Figure 5-2 Molecular Biology of the Cell 6e (© Garland Science 2015)

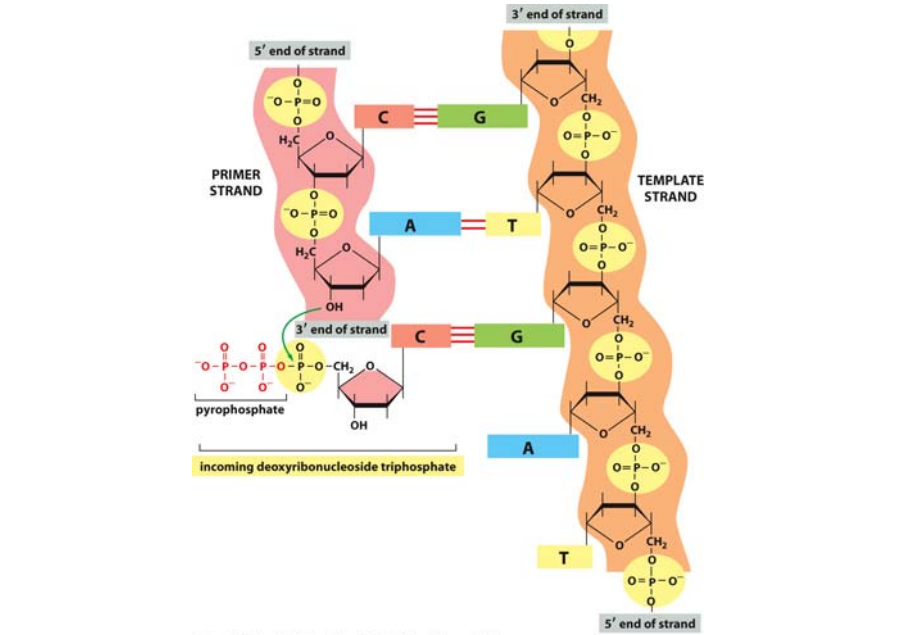
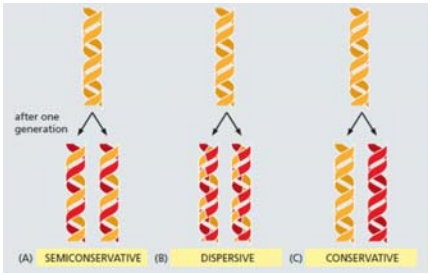
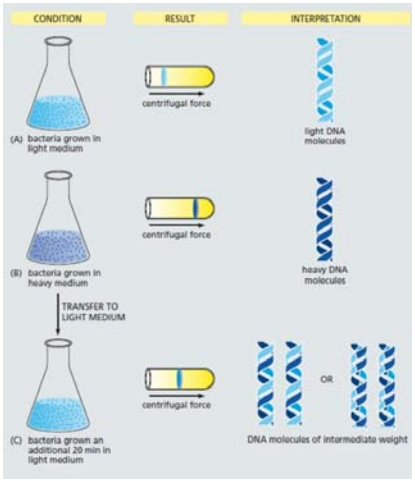


Figure 5-3 Molecular Biology of the Cell 6e (© Garland Science 2015)

Heat up DNA to find separate bands after 2nd generation: not dispersive.





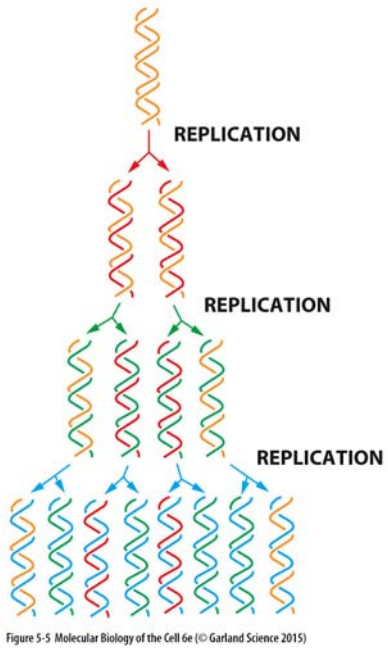


Figure 5-5 Molecular Biology of the Cell 6e (© Garland Science 2015)

DNA replication is bidirectional at each replication origin (slow in eukaryotes).

- Initiator proteins bind to replication origins, opening H bonds weak at ordinary temp
- Rep origins has AT (fewer H bonds than GC)
- One origin in bacteria, 10000 in humans
- Two replication forks one in each direction, 10x slower in humans due to chromatin
- DNA Polymerase adds nucleotides at 3' hydroxyl end of DNA chain to 5' phosphate of nucleotide using energy of deoxyribonucleoside triphosphate itself releasing pyrophosphate -> inorganic phosphate, irreversible reaction

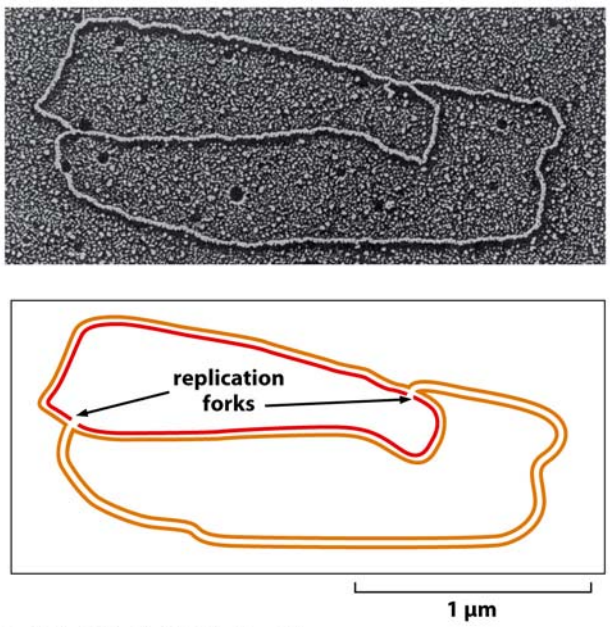


Figure 5-6 Molecular Biology of the Cell 6e (© Garland Science 2015)

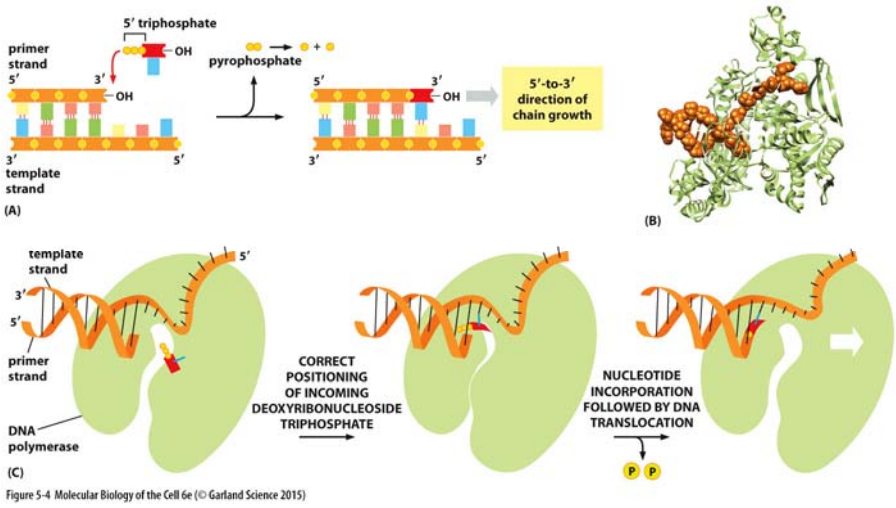


Figure 5-4 Molecular Biology of the Cell 6e (© Garland Science 2015)

DNA polymerase can correct its own errors before adding next nucleotide.

- Replication fork is asymmetric 5'->3', 3'->5'
- So 3'->5' is made discontinously and joined as Okazaki fragments (lagging strand)
- GT and AC less stable pairs 1/10^7 error
- DNA polymerase monitors nucleotide addition
- 3'->5' exonuclease proofreading: if incorrect, clips phosphodiester backbone using nuclease on diff catalytic domain (separate from post repair sys)
- Backstitching on works if polymer 5'->3' only

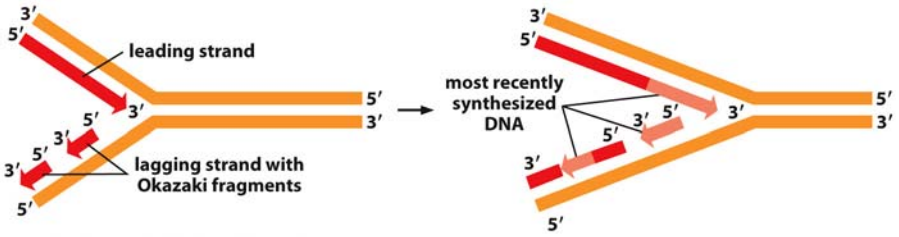


Figure 5-7 Molecular Biology of the Cell 6e (© Garland Science 2015)

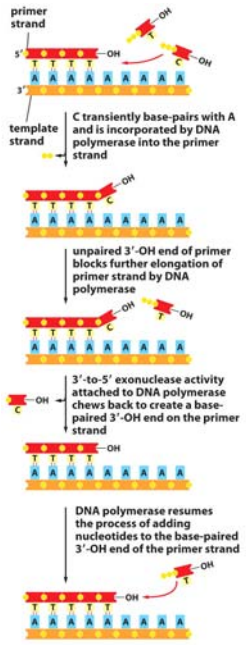


Figure 5-8 Molecular Biology of the Cell 6e (© Garland Science 2015)

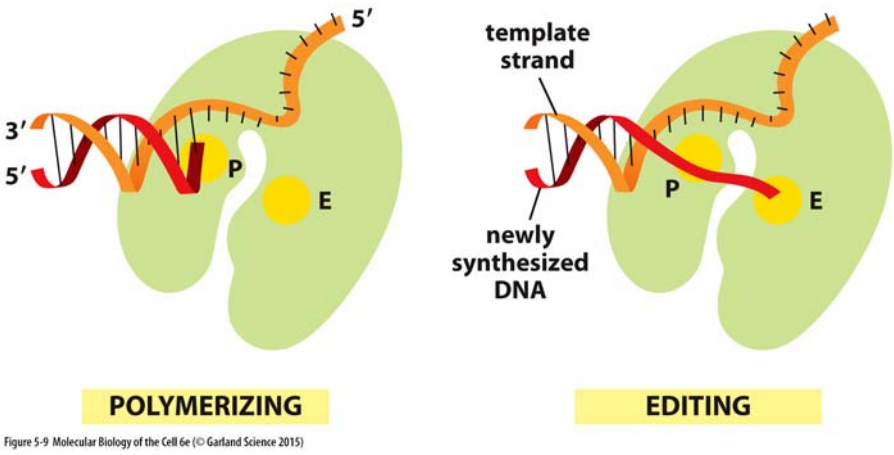


Figure 5-9 Molecular Biology of the Cell 6e (© Garland Science 2015)

Primase make ~10 nucleotide long RNA primers for DNA replication.

- Use U instead of T to make RNA from DNA
- Only one primer on leading, many primers on lagging, lets DNA poly begin new Okazaki frag, primers can have greater mutation rates
- Nuclease degrades RNA primer -> repair polymerase replaces RNA with DNA using Okazaki as primer -> DNA ligase joins DNA frag
- Primase does not proofread but DNA polymerases proofreads as RNA is replaced

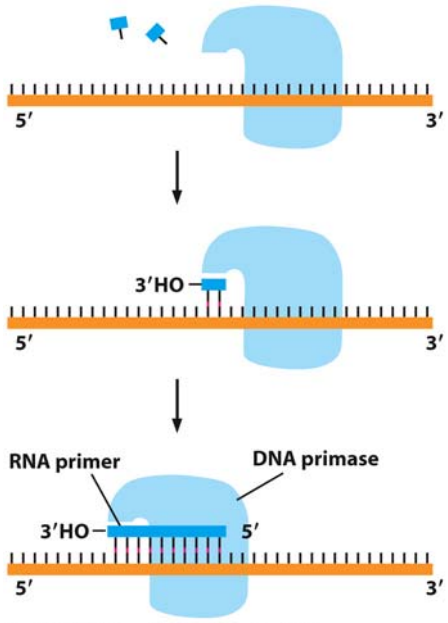


Figure 5-10 Molecular Biology of the Cell 6e (© Garland Science 2015)

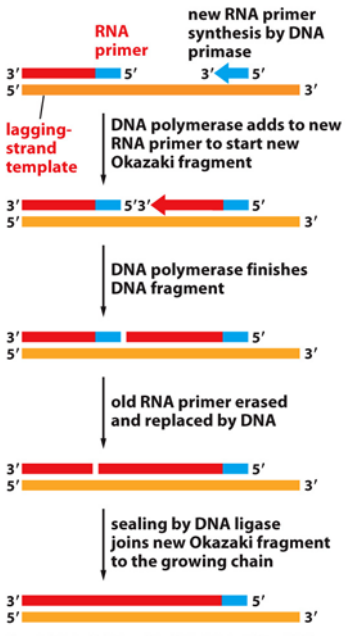


Figure 5-11 Molecular Biology of the Cell 6e (© Garland Science 2015)

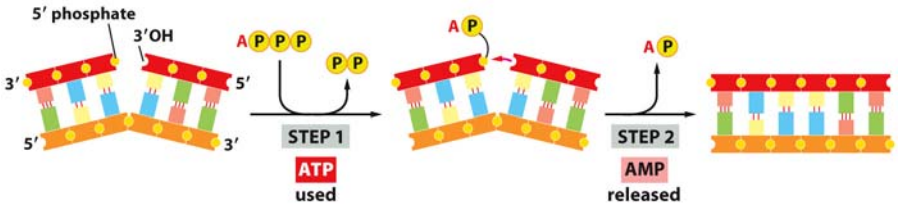


Figure 5-12 Molecular Biology of the Cell 6e (© Garland Science 2015)

Complex of replication machinery cooperate to synthesize DNA.

- DNA helicase uses ATP hydrolysis to pry apart double helix; Single-strand DNA-binding protein prevent base repairing holds template elongated
- DNA topoisomerase relieves tension of excess twisting in front of replication fork using temp nicks in backbone, and untangle intertwined DNA
- Sliding clamp forms ring on new helix, keeps DNA polymerase on template w/o falling off
- Clamp loader locks in clamp using ATP, loads each time a new Okazaki fragment is begun

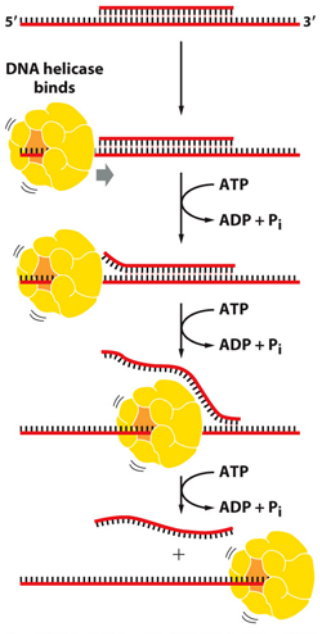


Figure 5-13 Molecular Biology of the Cell 6e (© Garland Science 2015)

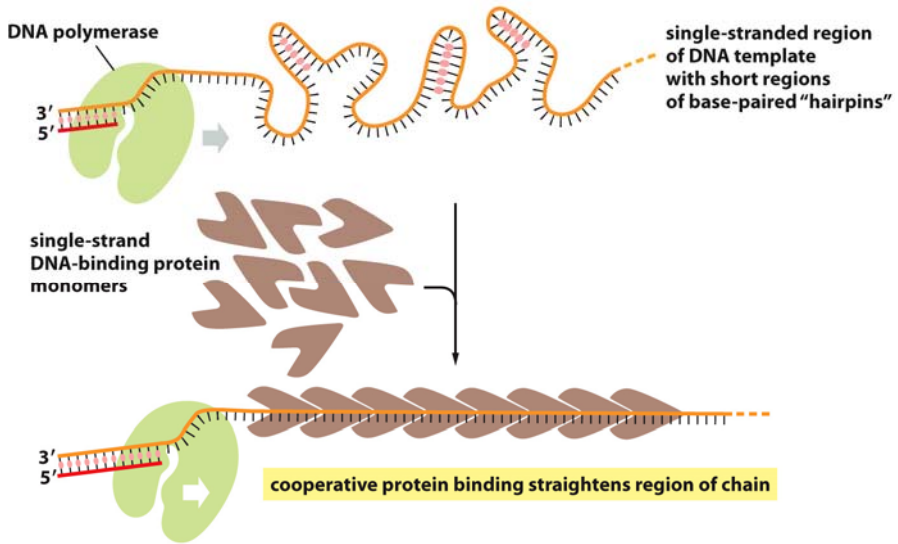


Figure 5-15 Molecular Biology of the Cell 6e (© Garland Science 2015)

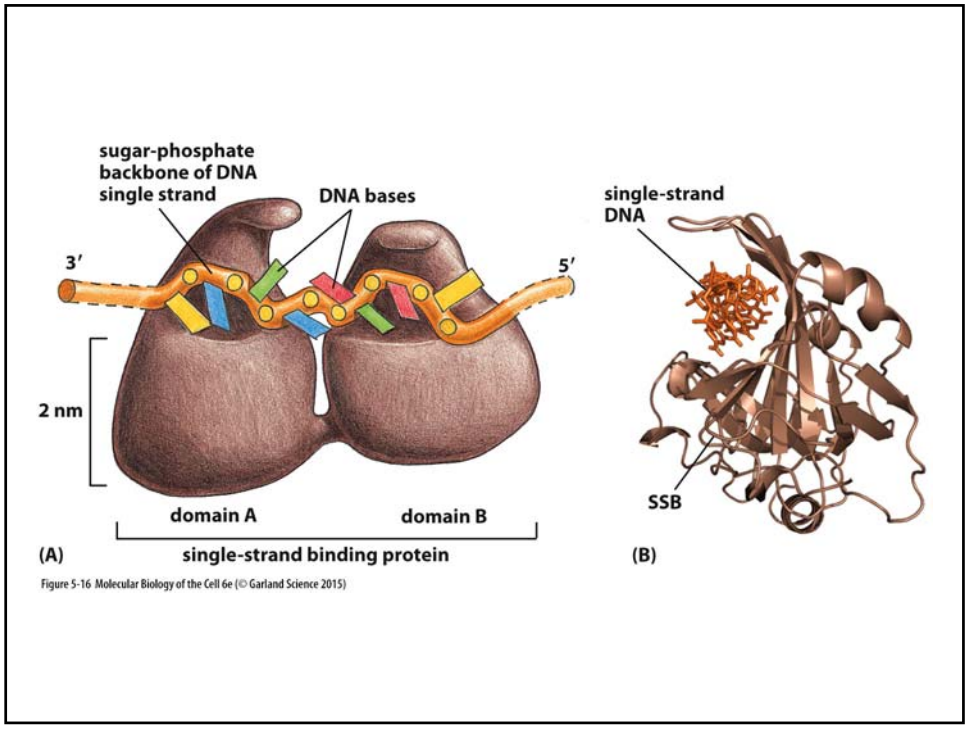
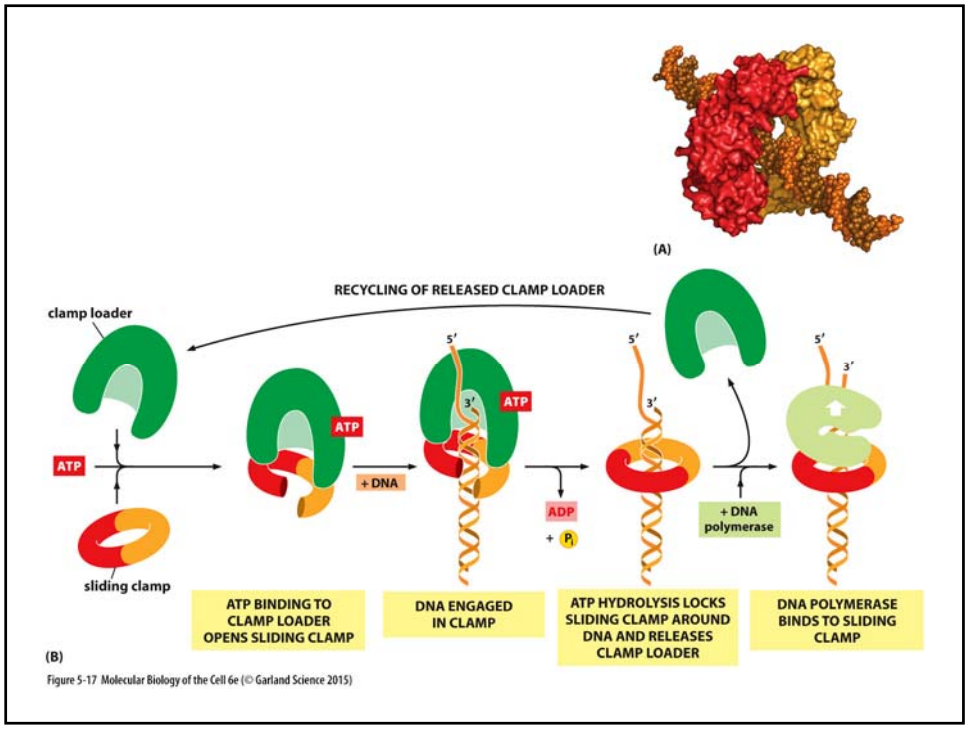


Figure 5-16 Molecular Biology of the Cell 6e (© Garland Science 2015)



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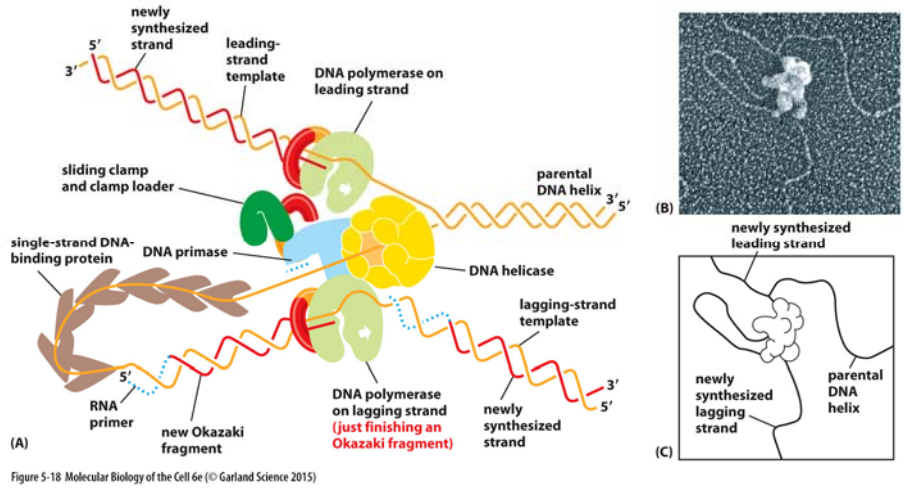

Figure 5-18 Molecular Biology of the Cell 6e (© Garland Science 2015)

Telomeres are noncoding long repeats at the ends of chromosomes.

- DNA replication only 5'->3' leaves lagging strand short of completion
- Bacteria solves problem using circular DNA, eukaryotes have repeat sequences at ends
- Telomerase adds copies of DNA repeats to template strand using RNA as template
- Telomeres allow recognition of natural ends of chromosomes distinct from accidental breaks

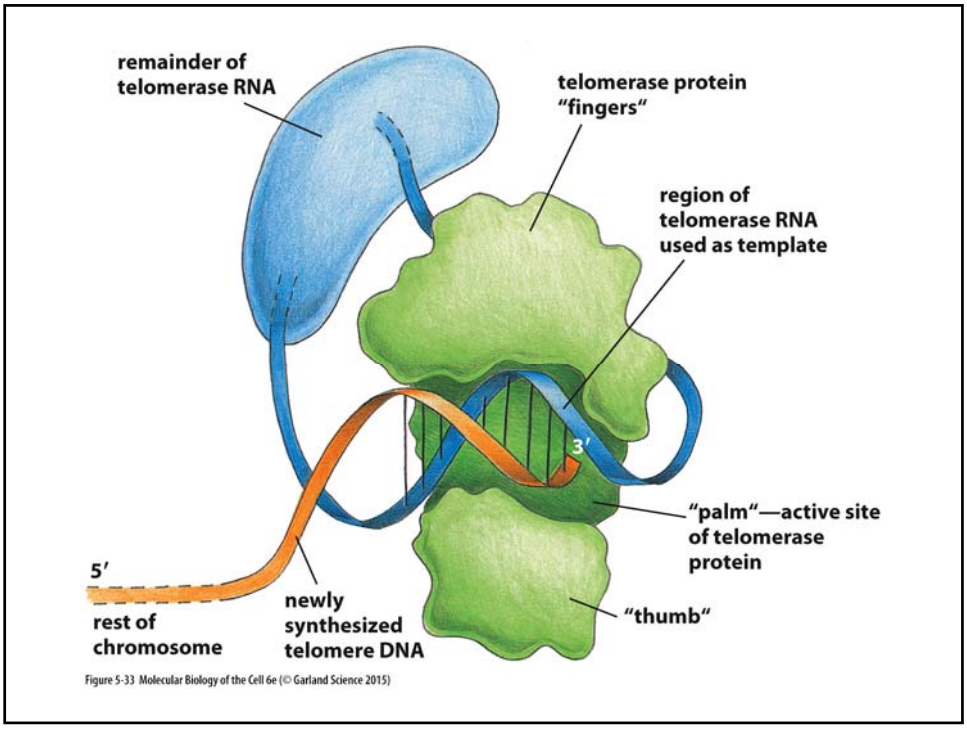

Figure 5-33 Molecular Biology of the Cell 6e (© Garland Science 2015)

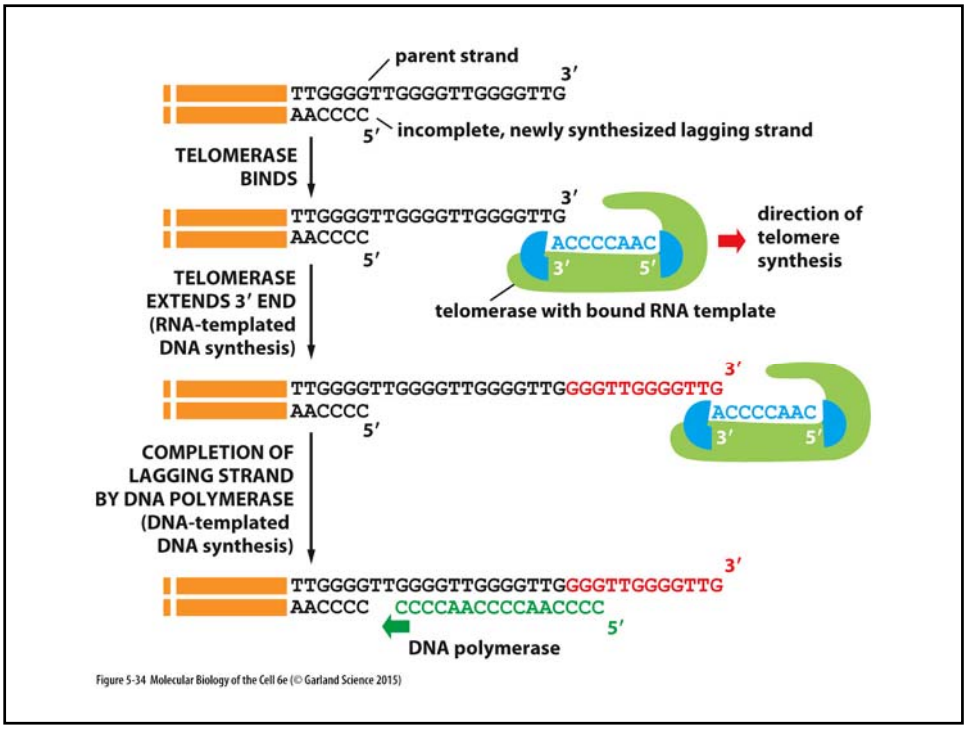

Figure 5-34 Molecular Biology of the Cell 6e (© Garland Science 2015)

Team work

During DNA replication in the cell, DNA primase makes short primers that are then extended by the replicative DNA polymerases. These primers …

- A. provide a 3'-phosphate group for the DNA polymerases to extend.
- B. are made up of DNA.
- C. are made more frequently in the leading strand than the lagging strand.
- D. are joined in unmodified form to the neighboring DNA.
- E. generally have a higher number of mutations compared to their neighboring DNA.

The telomerase enzyme in human cells …

- A. creates the "end-replication" problem.
- B. polymerizes the telomeric DNA sequences without using any template.
- C. removes telomeric DNA from the ends of the chromosomes.
- D. has an RNA component.
- E. extends the telomeres by its RNA polymerase activity.