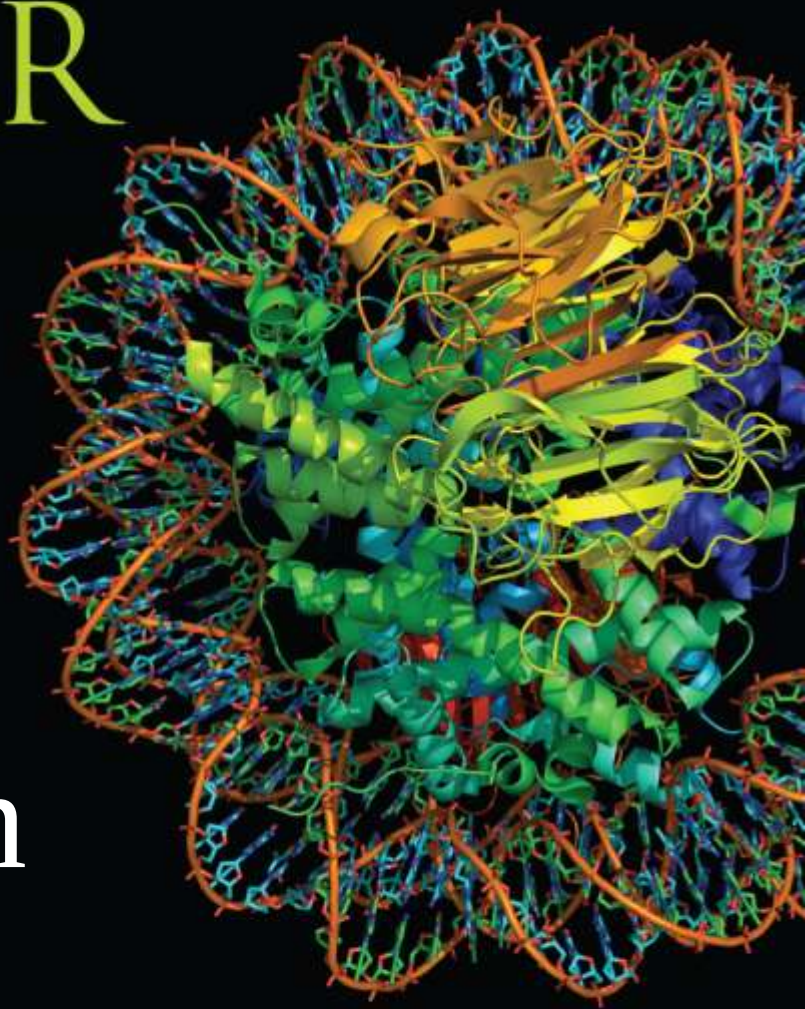
Principles of

MOLECULAR BIOLOGY

BURTON E. TROPP



Chapter 8
DNA Replication

General Features of DNA Replication

DNA replication is semi-conservative

- 2 parental strands separate allowing each separated strand to serve as a template
- Each daughter molecule has one parental strand and one newly synthesized strand

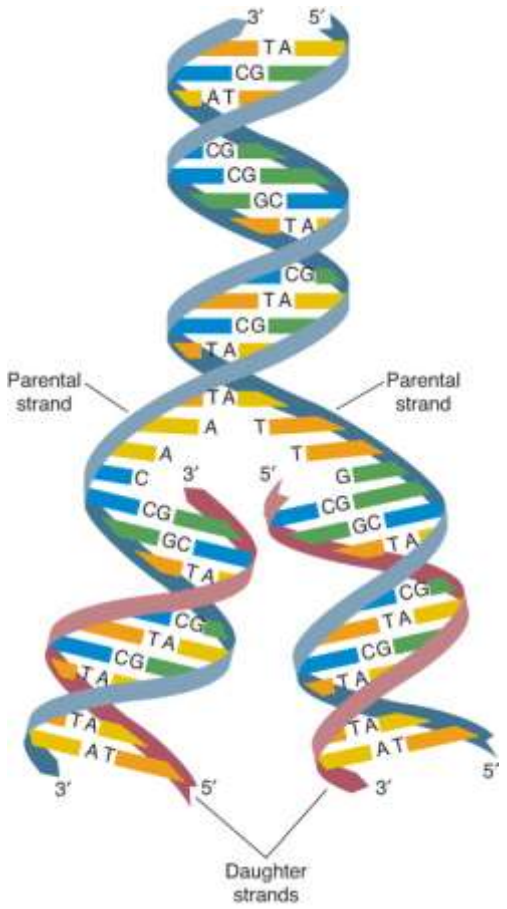


Figure 08.01: Watson-Crick model of DNA replication.

General Features of DNA Replication

- 2 alternative models
  - Conservative replication
  - Dispersive Replication

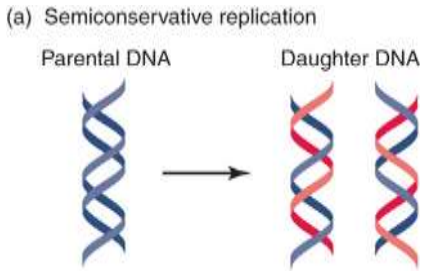


Figure 08.02A: Three models of replication. (a) Semiconservative DNA replication.

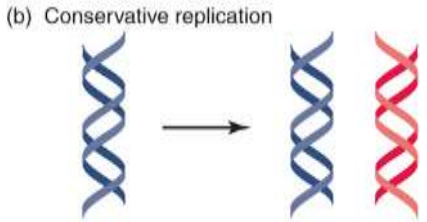


Figure 08.02B: Three models of replication. (b) Conservative DNA replication.

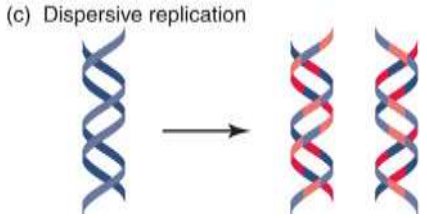


Figure 08.02C: Three models of replication. (c) Dispersive DNA replication.

General Features of DNA Replication

- Conservative Replication
  - Parental strands unwind at replication site to allow the sequence to be read by the DNA polymerase and then rewinds
  - One of the 2 molecules contains both original strands
  - Other is made of 2 new strands

General Features of DNA Replication

- Dispersive Replication
  - Each strand of the daughter molecules is interspersed with sections of both old and new DNA

General Features of DNA Replication

Meselson and Stahl experiment

- Used ^{15}N heavy nitrogen isotope to label bacterial DNA
- After uniform labeling bacteria were transferred to a "light" medium with ^{14}N
  - Removed samples at different times
  - Extracted DNA
  - Separated molecules using CsCl equilibrium density centrifugation
- Semi-conservative replication model is the only one consistent with results

General Features of DNA Replication

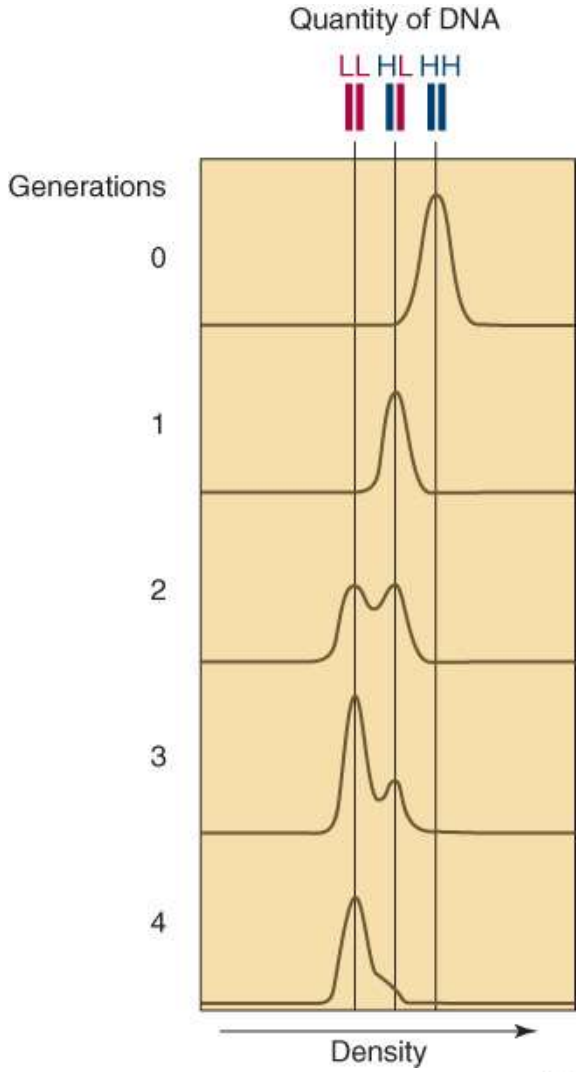


Figure 08.03: Meselson-Stahl experiment showing semiconservative DNA replication.

General Features of DNA Replication

- Bacterial and eukaryotic DNA replication is bidirectional
  - θ-structure can be explained by unidirectional or bidirectional replication
  - Labeling expt. confirmed that it is bidirectional

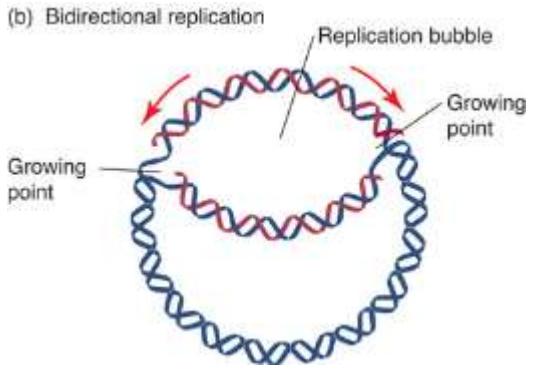


Figure 08.05A: Two alternate methods of replication. (a) In unidirectional replication a single growing point moves in the direction shown by the red arrow.

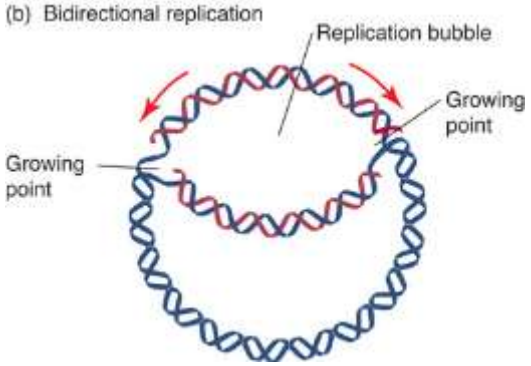


Figure 08.05B: Two alternate methods of replication. (b) In bidirectional replication two growing points move in opposite directions, as shown by the red arrows.

General Features of DNA Replication

Bidirectional replication means that one chain grows 5’→3’ but other strand is 3’→5’

– Problem: No DNA polymerase catalyzes in that direction

- The DNA strand that grows in an overall 3’→5’ direction is formed by joining short fragments

General Features of DNA Replication

In 1968 Reiji Okazaki proposed 2 models

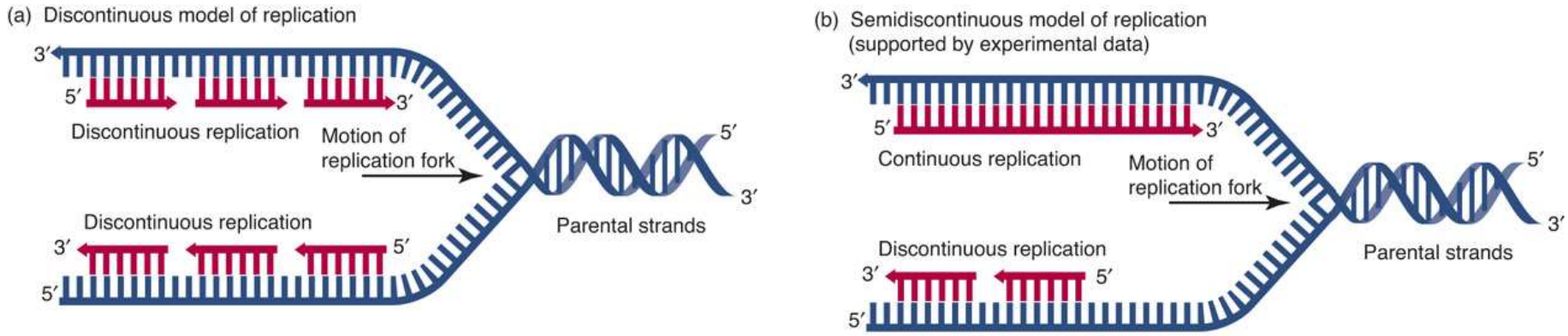


Figure 08.07: Models proposed by Reiji Okazaki to explain in vivo DNA replication. Parental strands are shown in blue and newly replicated strands are shown in red.

General Features of DNA Replication

- Semi-discontinuous replication
  - Forms Okazaki fragments
  - Lags behind the continuously replicating strand
    - Leading strand
    - Lagging strand
- DNA ligase connects adjacent Okazaki fragments

General Features of DNA Replication

RNA serves as a primer for Okazaki fragment synthesis

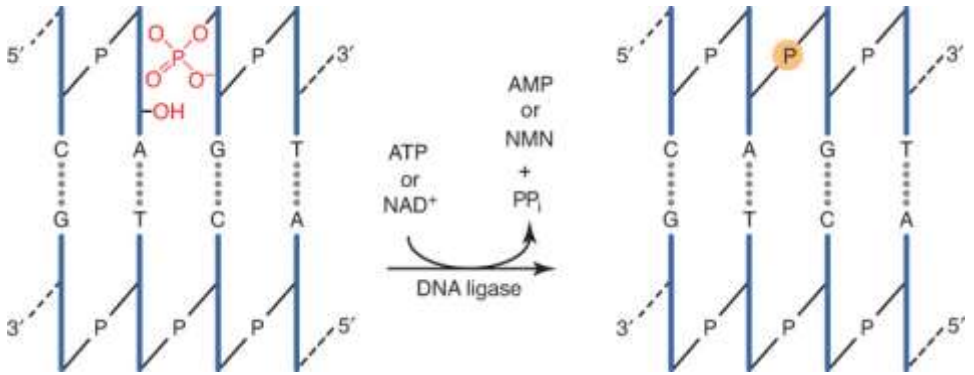


Figure 08.11: RNA primers for Okazaki fragments. The synthesis of Okazaki fragments is primed by short RNA segments.

General Features of DNA Replication

Processing Okazaki fragments

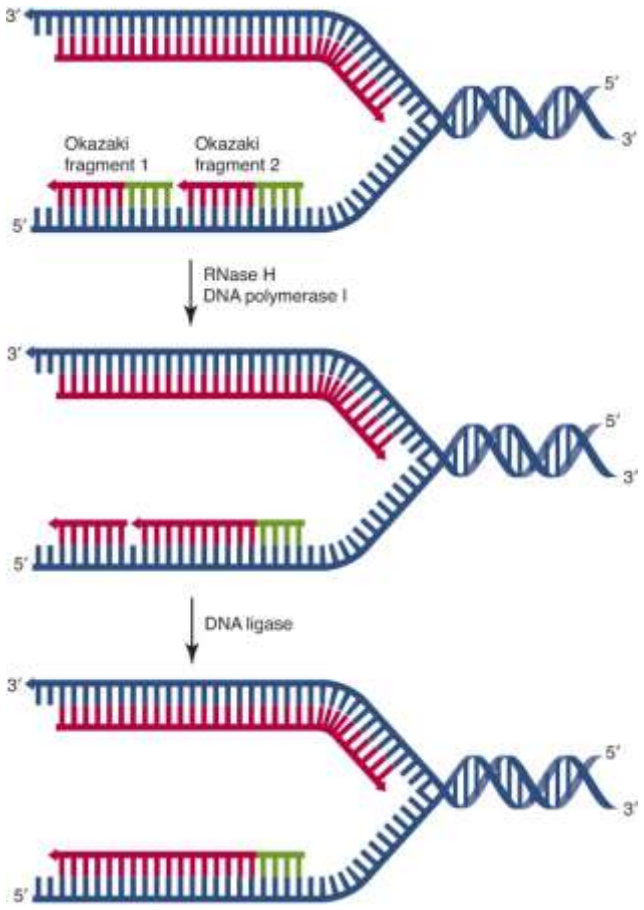


Figure 08.12: Pathway for processing Okazaki fragments.

Bacterial DNA Replication

- E. coli replication apparatus stages
  - Initiation: the assembly of the replication apparatus at a unique site
  - Elongation: leading strand is synthesized continuously. Lagging strand is synthesized discontinuously
  - Termination: the 2 replication forks meet about halfway around the bacterial chromosome

Bacterial DNA Replication

Replicon Model

2 specific components: initiator protein and a replicator (a specific set of DNA sequences)

- Specific site at which replication is initiated
  - Origin of replication (*ori*)
  - Eukaryotic chromosomes require many origins of replication

Bacterial DNA Replication

- E. coli chromosomal replication begins at *oriC*
  - Right side of sequence
    - Five 9bp sites (R1-R5) known as Dna A boxes
  - Left side of sequence
    - DNA unwinding element (DUE) with three 13bp AT-rich elements
  - Multiple GATC site

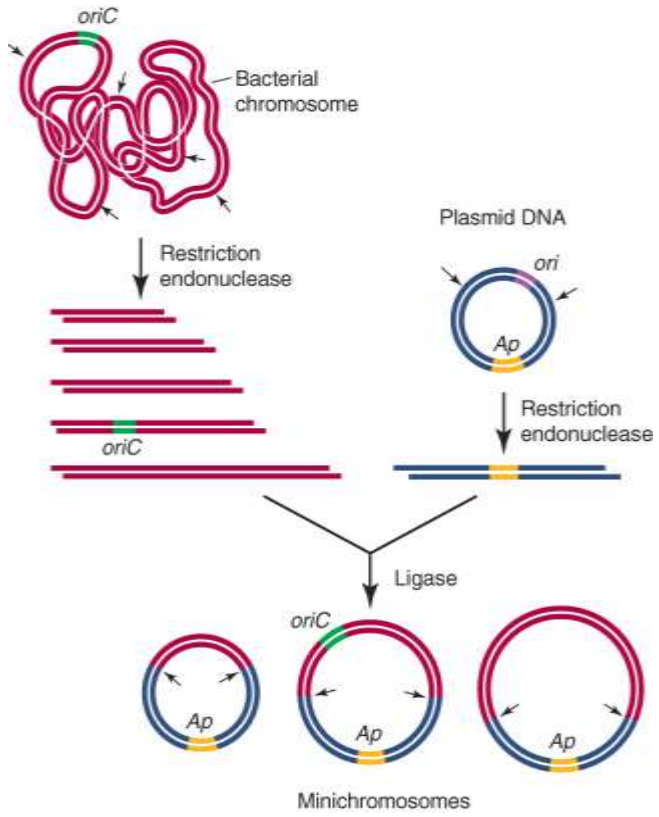


Figure 08.13: Isolation of the E. coli replication origin oriC.

(Adapted from Kornberg, A., and Baker, T. A. 1991. DNA Replication (2nd ed). W. H. Freeman and Company.)

Bacterial DNA Replication

- Four proteins are critical in the DNA replication initiation process
- DnaA – initiator
- DnaB – helicase
- DnaC – loader
- DnaG - primase

Bacterial DNA Replication

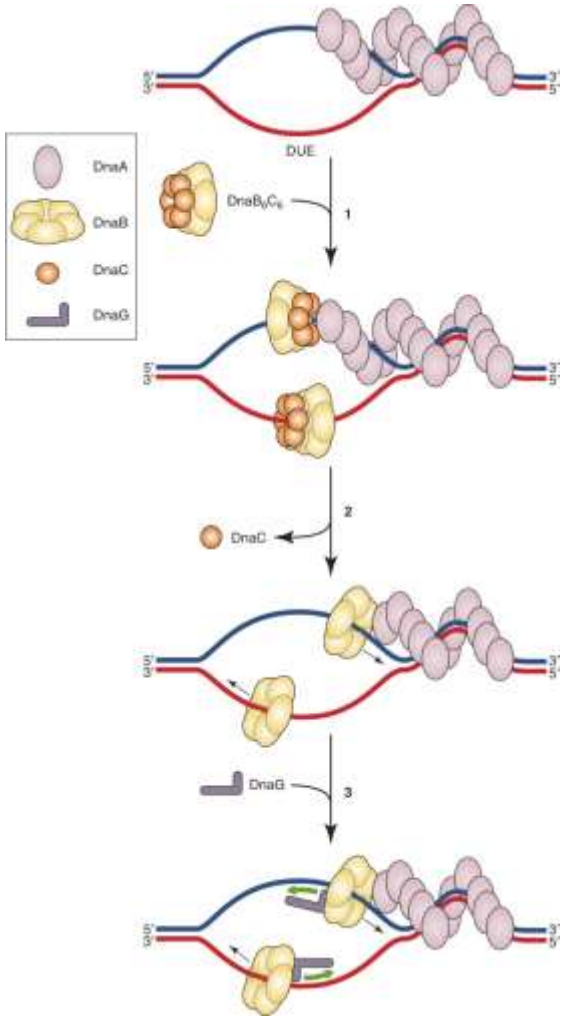


Figure 08.15: Model for loading DnaB onto single strands at DUE.

(Adapted from Mott, M. et al. 2008. Cell 134:623–634.)

Bacterial DNA Replication

Several enzymes act together at the replication fork

- DNA polymerase I-required
- DNA polymerase II-not essential
- DNA polymerase III-required

Bacterial DNA Replication

- Purified DNA polymerase III is very slow and dissociates from its DNA frequently
  - Enzymes that remain tightly associated with their template are said to be **highly processive**

Bacterial DNA Replication

- DNA polymerase III holoenzyme has three distinct subassemblies
  - Core polymerase
  - Clamp loader
  - Sliding clamp

Bacterial DNA Replication

- The core polymerase has one subunit with 5’ 3” polymerase activity and another with 3’ 5’ exonuclease activity
  - Core polymerase (previously DNA PolIII)
    - α-subunit
      - 5’ →3’ chain growth
    - ε-subunit
      - 3’ →5’ exonuclease activity
    - θ-subunit
      - Stimulates ε-subunit but not essential

Bacterial DNA Replication

The sliding clamp forms a ring around DNA, tethering the remainder of the polymerase holoenzyme to the DNA

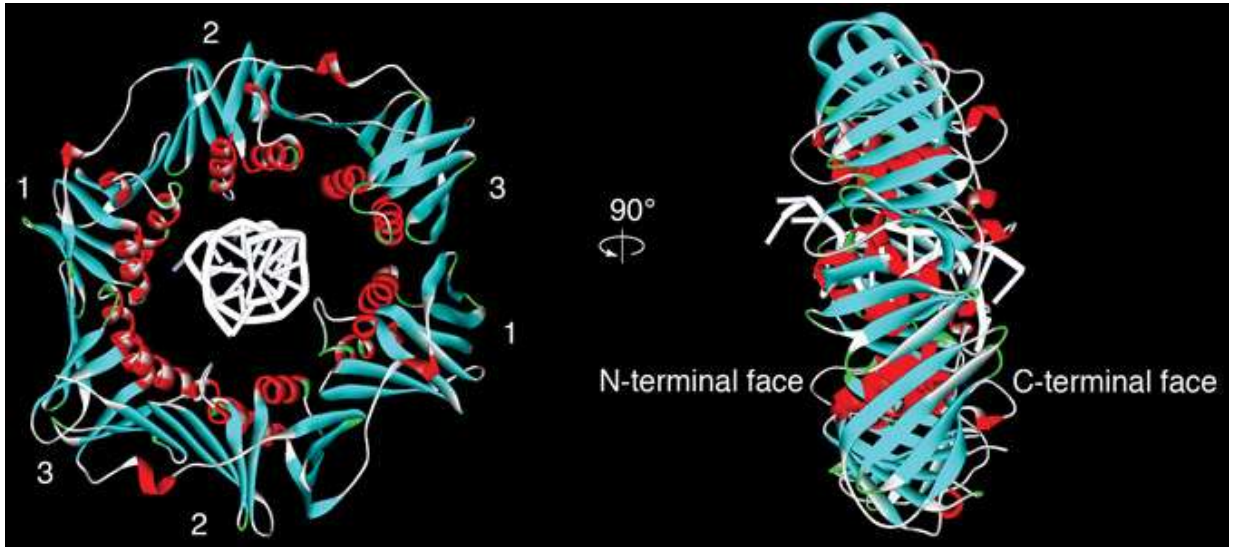


Figure 08.18: Structure of an E. coli sliding clamp on DNA. The two subunits are shown as solid ribbons. α-Helices are red, β-structures are cyan, and turns are white.

(Protein Data Bank ID: 3BEP. Georgescu, R. E., et al. 2008. Cell 132:43–54.)

Bacterial DNA Replication

The clamp loader places the sliding clamp around DNA

- Uses energy provided by ATP to load the sliding clamp onto a DNA template-primer with a 5’ overhang

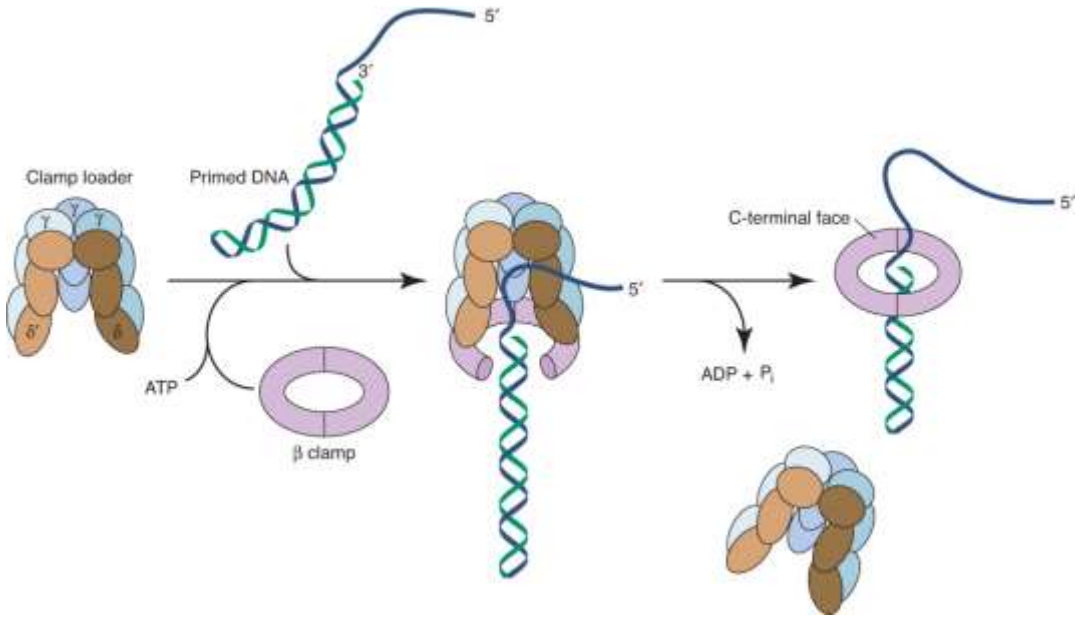


Figure 08.19: The clamp loading cycle.

(Adapted from Pomerantz, R. T., and O’Donnell, M. 2007. Trends Microbiol 15:156–164.)

Bacterial DNA Replication

The replisome catalyzes coordinated leading and lagging strand DNA synthesis

- The trombone model of replication
- Lagging strand loops out
  - Allows the pair of core polymerases to coordinate leading and lagging strand synthesis
- Requires
    - DNA Pol III holoenzyme
    - Helicase
    - Primase
    - Single-stranded DNA binding protein
- This is the **replisome**

Bacterial DNA Replication

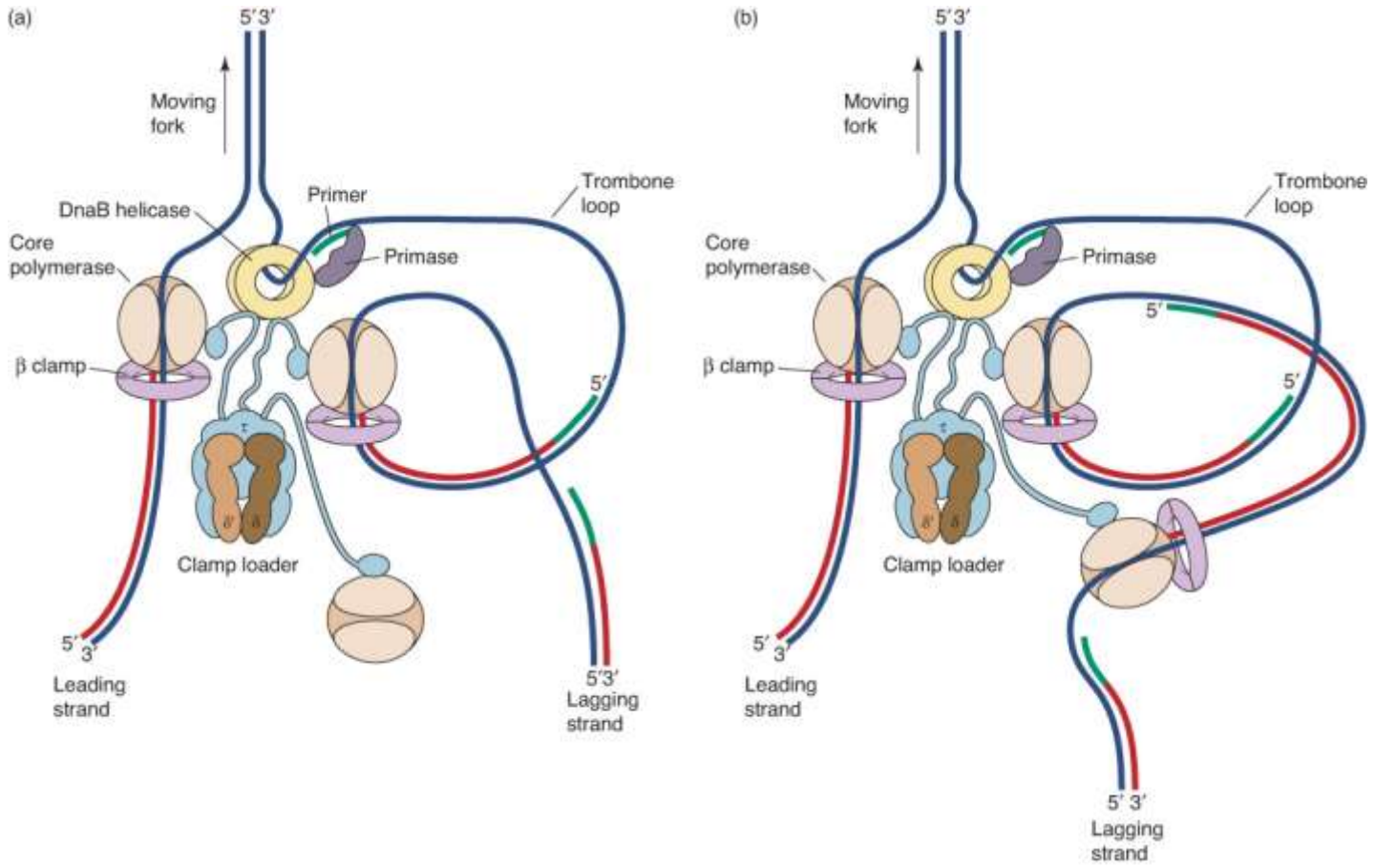


Figure 08.20: DNA replisome with three core polymerases.

(Adapted from McInerney, P. et al. 2007. Mol Cell 27:527–538.)

Bacterial DNA Replication

There are 3 steps for lagging strand synthesis

1. Core polymerase extends the Okazaki fragment and clamp loader loads on a new sliding clamp
2. Core polymerase dissociates upon reaching an adjacent double-stranded duplex
3. Core polymerase binds to the new sliding clamp and synthesizes DNA

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

Bacterial DNA Replication

E. coli DNA replication terminates when the two growing forks meet in the terminus region

- Terminus utilization substance (Tus) binds to the termination site (Ter site)
  - Arrests the progress of the replication fork

Topisomerase IV and recombinase separate newly formed sister chromosomes

Bacterial DNA Replication

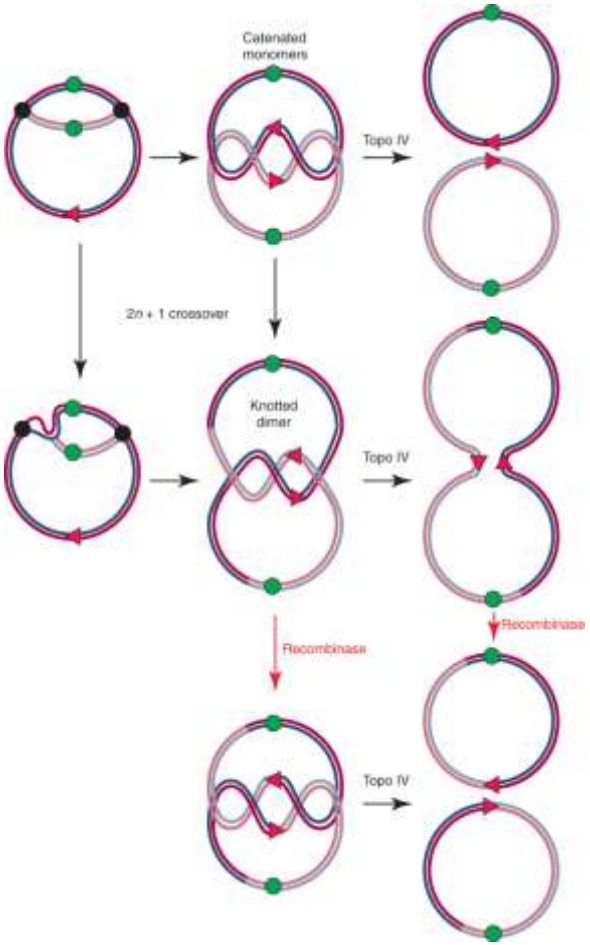


Figure 08.24: Links between replication, recombination, and chromosome segregation.

(Adapted from Sherratt, D.J., Lau, I.F., and Barre, F.X. 2001. Curr Opin Microbiol 4:653–659.)

Eukaryotic DNA Replication

The SV40 DNA replication system is a good model for *in vitro* eukaryotic DNA replication

- 6 large T antigen molecules form a helicase that interacts with the origin of replication
  - Recruits DNA polymerase α-primase (Pol α)
  - Forms RNA primers 8-12 nucleotides long then incorporates 15-25 deoxyribonucleotides before dissociating
    - **Pol α generates an RNA primer joined to a short DNA segment (initiator DNA)**

Eukaryotic DNA Replication

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
   - Eukaryotic clamp loader
4. Flap endonuclease (FEN1)
   - Acts during Okazaki fragment maturation
5. DNA ligase
   - seals the nick between adjacent Okazaki fragments

Eukaryotic DNA Replication

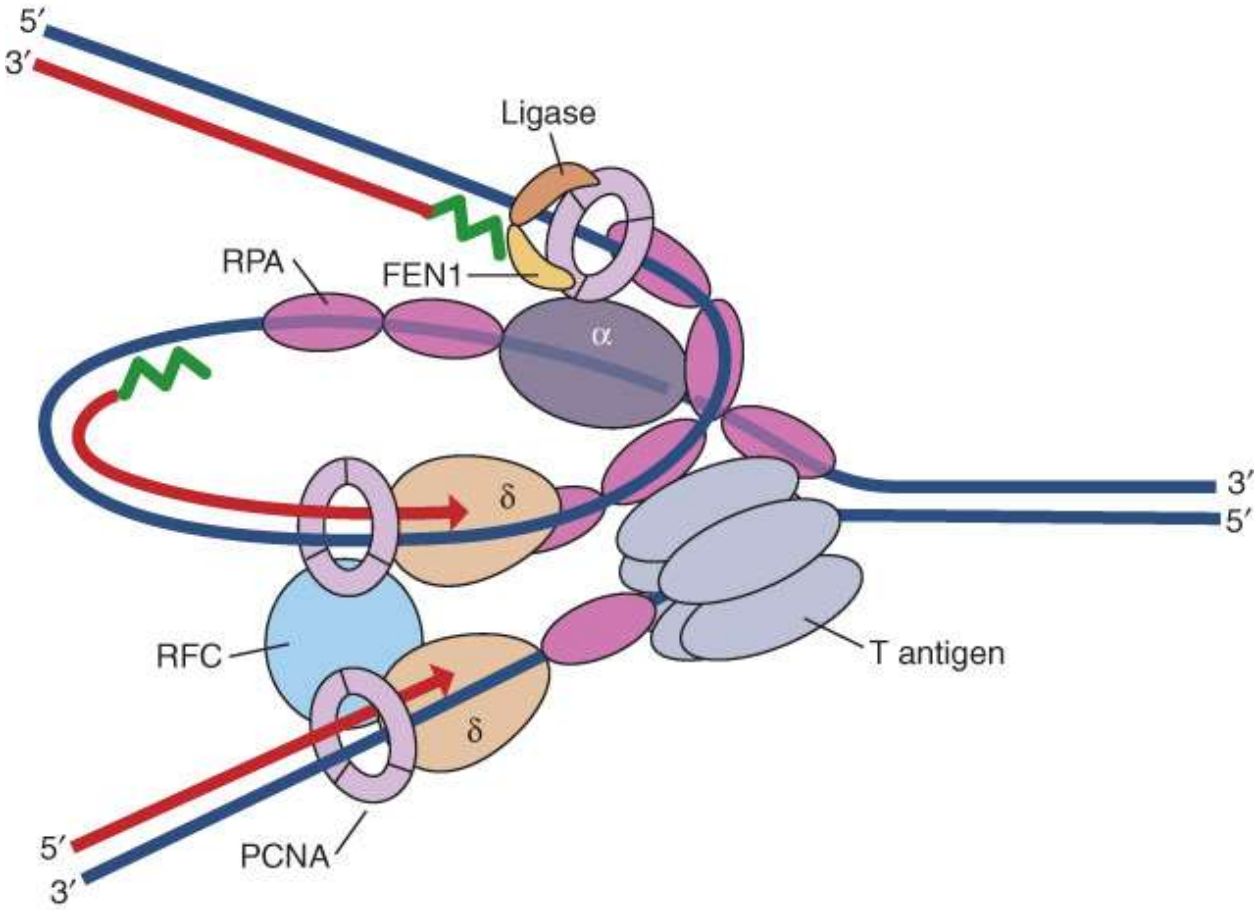


Figure 08.27: Model for the organization of proteins and enzymes at SV40 replication fork.

(Adapted from Stillman, B. 2008. Mol Cell 30:259–260.)

Eukaryotic DNA Replication

Eukaryotic replication machinery must replicate long linear duplexes with multiple origins of replication

- Requires multiple origins of replication
- Requires 3 different DNA polymerases
- Linear eukaryotic chromosomes require telomerase to form their ends

Eukaryotic DNA Replication

Eukaryotic DNA replication initiates at multiple sites

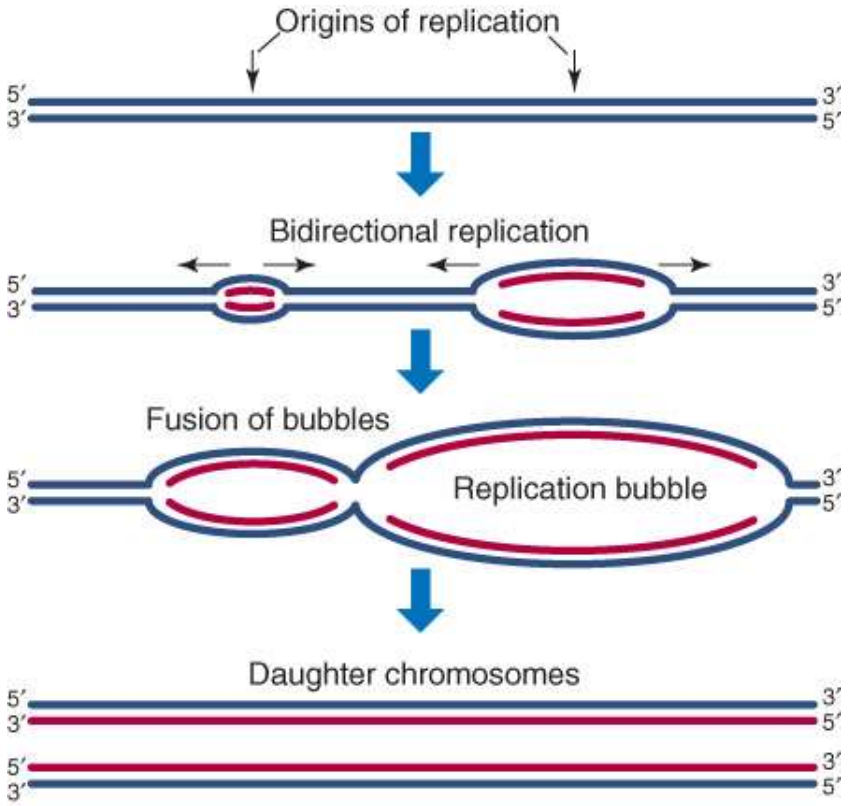


Figure 08.29: Schematic showing the fusion of a pair of replication bubbles.

Eukaryotic DNA Replication

Eukaryotic cells must ensure that each origin fires once and only once in each cell cycle

- Origin Recognition Complex (ORC)
  - 6 protein subunits
  - When bound to DNA recruits Cdc6 which is degraded once initiation is completed

Eukaryotic DNA Replication

Three DNA polymerases are necessary for eukaryotic replication

1. Pol α
   - Responsible for forming RNA primer and initiator DNA
2. Pol δ
   - Responsible for lagging strand synthesis
3. Pol ε
   - Responsible for leading strand synthesis

Eukaryotic DNA Replication

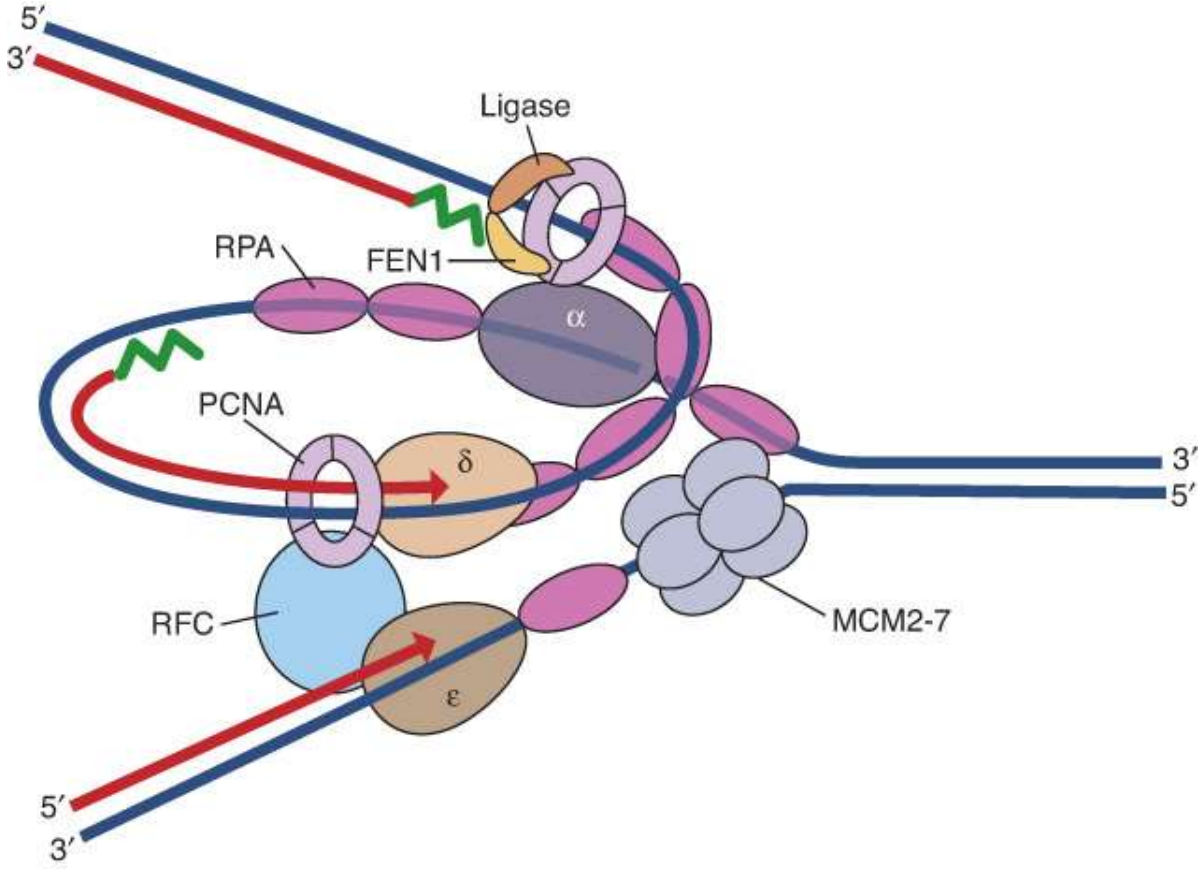


Figure 08.33: Simplified model for the organization of proteins and enzymes at the eukaryotic replication fork.

(Adapted from Stillman, B. 2008. Mol Cell 30:259–260.)

Eukaryotic DNA Replication

A terminal transferase-like enzyme is required for telomere formation

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

Eukaryotic DNA Replication

Telomerase uses an RNA template to add nucleotide repeats to chromosome ends

- Contains an RNA that acts as a template for telomeric DNA synthesis.
  - Acts as an RNA dependent DNA polymerase that supplies its own template RNA

Eukaryotic DNA Replication

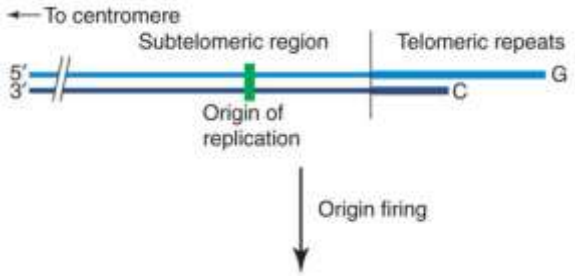


Figure 08.37A: Leading strand problem. (a) The telomere G-rich strand ends in a 3′-single strand that overhangs the C-rich strand.

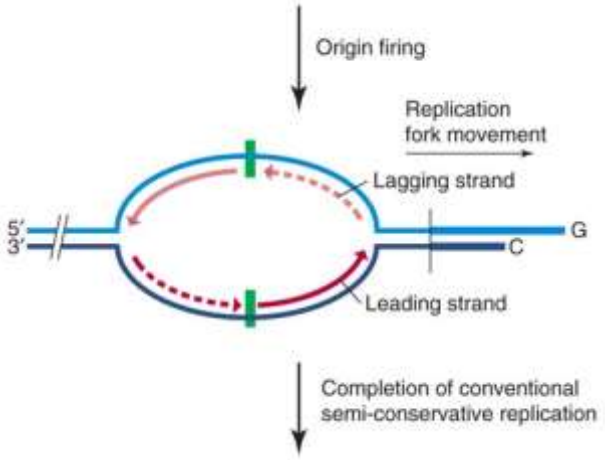


Figure 08.37B: Leading strand problem. (b) Semiconservative replication initiates at origins internal to the telomeric repeats.

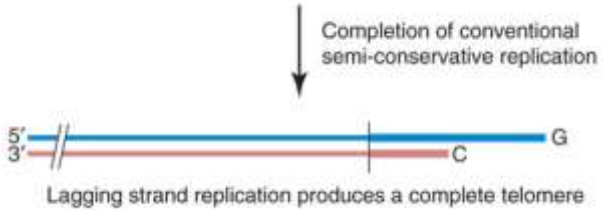


Figure 08.37C: Leading strand problem. (c) Synthesis of lagging strands does not necessarily present a problem for the replication machinery.

Figure 08.37D: Leading strand problem. (d) Synthesis of leading strands introduces a problem because replication stops at the 5′-end of the C-rich template.

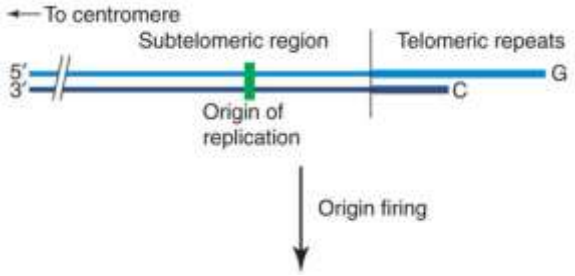
(Adapted from Chakhparonian, M., and Wellinger, R.J. 2003. Trends Genet 19:439–446.)

Eukaryotic DNA Replication

Telomerase plays an important role in solving the end-replication problem

- Leading strand replication presents a serious problem
  - Leading strand replication produces a blunt end with a loss of sequence information

Eukaryotic DNA Replication



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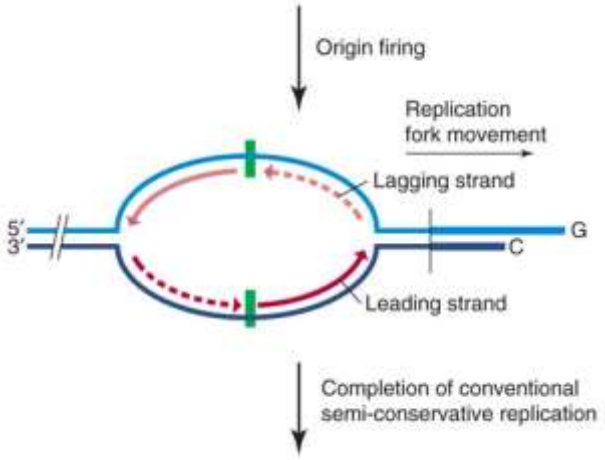

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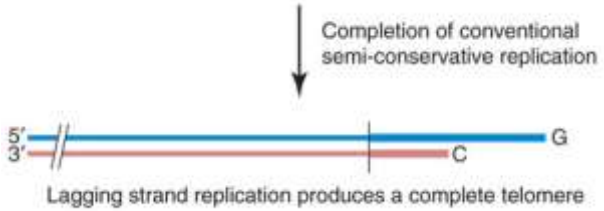

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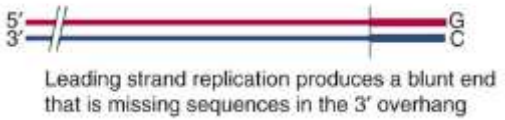

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(Adapted from Chakhparonian, M., and Wellinger, R.J. 2003. Trends Genet 19:439–446.)

Eukaryotic DNA Replication

Telomerase function

1. A strand specific DNA 5’→3’ exonuclease removes nucleotides to generate an overhang
2. Telomerase extends the 3’ overhang
3. Newly synthesized region serves as a template for standard replication, restoring the 5’ end 0

Eukaryotic DNA Replication

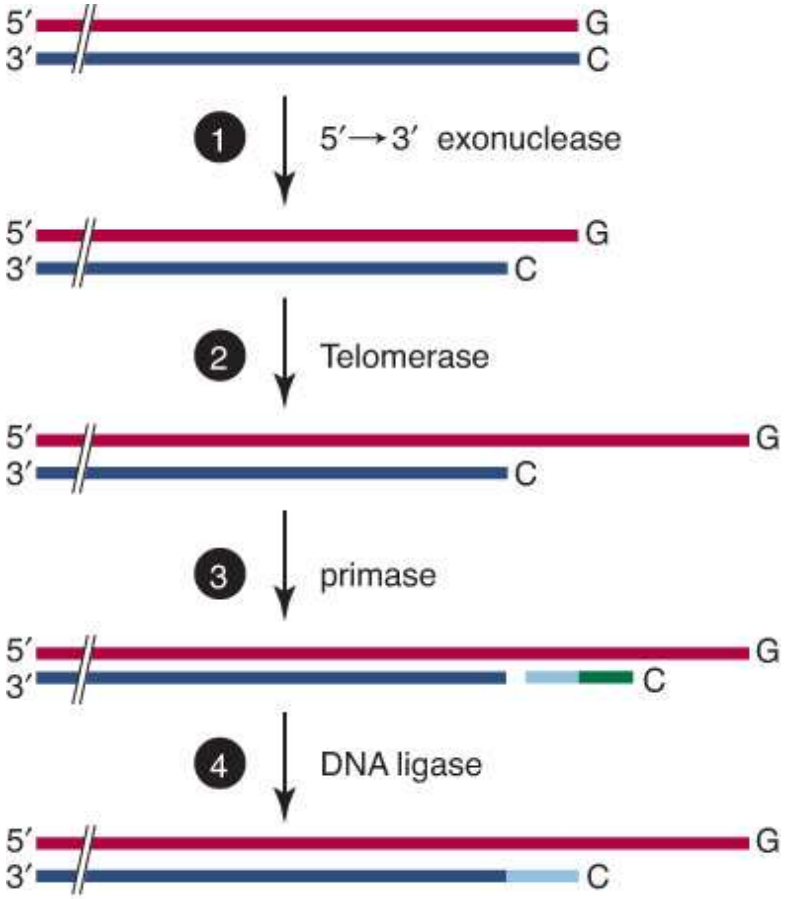

Figure 08.38: Hypothesis for solving the leading strand problem.

Replication Coupled Chromatin Synthesis

Chromatin disassembly and reassembly are tightly coupled to DNA replication

- The nucleosome blocks direct contact between the DNA synthesis machinery and its DNA substrate
- ≈300bp of nucleosome-free (naked) DNA is present just before the replication fork
- ≈250bp of naked DNA is present just after the replication fork

Replication Coupled Chromatin Synthesis

- Histone chaperones permit ordered chromatin disassembly or assembly
- Chromatin modifiers also participate by adding acetyl, methyl, phosphate or other groups to histones or removing them from modified histones