Lecture 8: DNA replication

Learning objectives

- Compare the roles of DNA polymerases in E. coli with those in mammalian cells
- Contrast the mechanisms of synthesis of the leading and lagging strands of DNA
- Identify the proteins found at replication forks of bacteria and mammalian cells
- Describe the mechanisms that ensure accurate DNA replication
- Compare origins of replication in bacteria and mammalian cells
- Summarize the action of telomerase

DNA polymerases

All DNA polymerase catalyzes the 5’ to 3’ joining of deoxyribonucleoside triphosphates (dNTPs)

Bacteria DNA polymerases:

- Pol I: removal of RNA primers
- Pol II: replication of damaged DNA
- Pol III: the main DNA polymerase

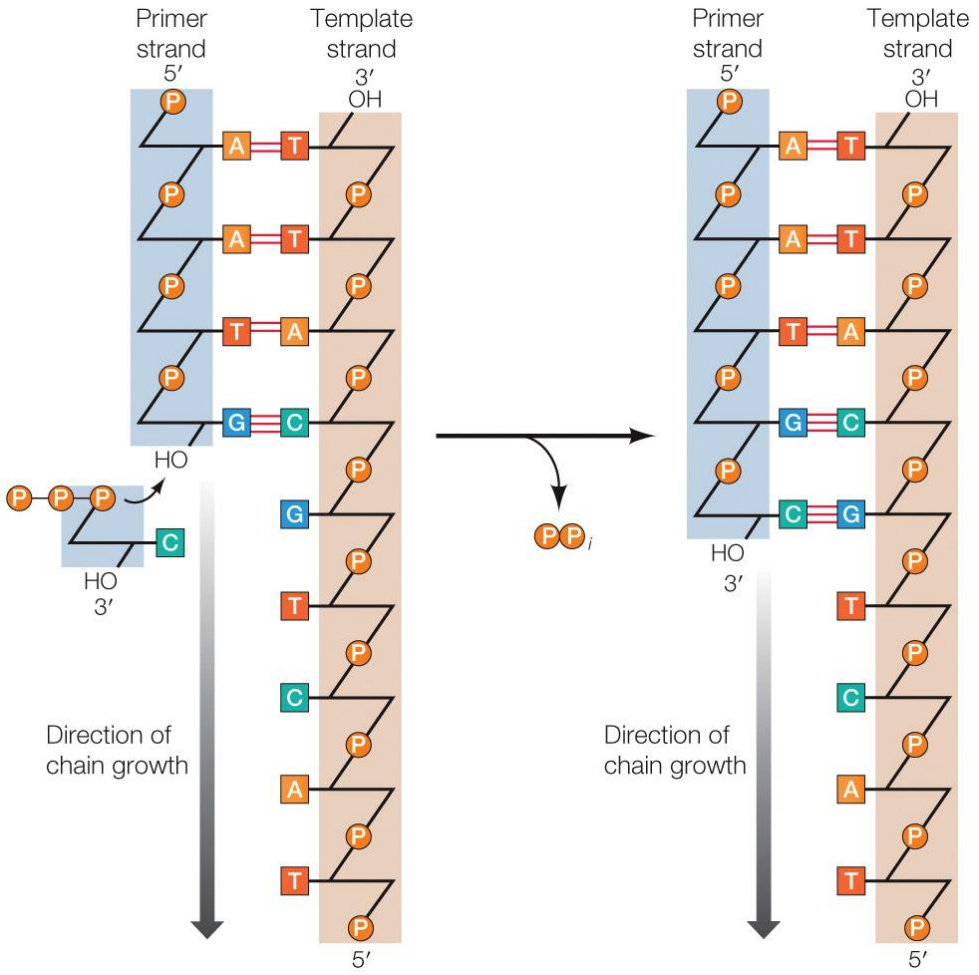
Eukaryote DNA Polymerases:

- Pol α: primase (synthesizing RNA primer)와 complex 형성하여 elongating primer
- Pol β: repairing DNA.
- Pol γ: replicating mitochondrial DNA
- Pol δ & Pol ε: the main polymerases

Two fundamental properties of all DNA polymerases

1. All DNA polymerases synthesize DNA only in the 5’ to 3’ direction.
2. DNA polymerases can add a new nucleotide only to a preformed primer strand that is hydrogen-bonded to the template.

→ Proofreading ability; critical for maintaining the high fidelity of DNA replication;

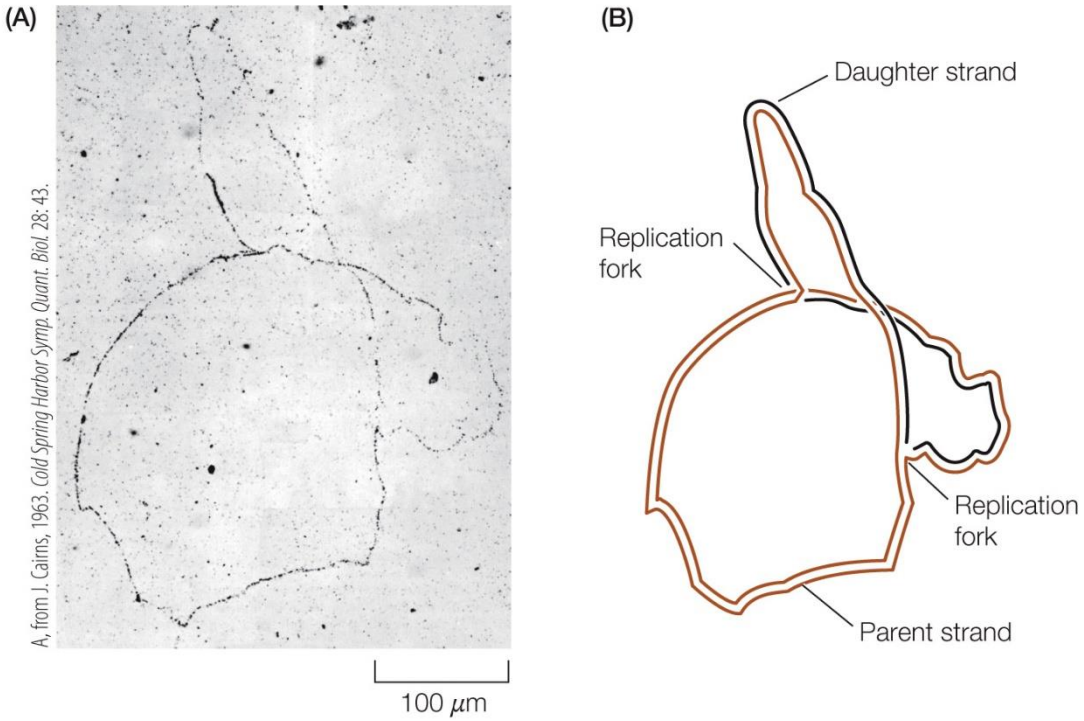


Replication Fork (복제분기점)

John Cairns, radioactive thymidine의 존재하에서 *E.coli*를 배양하여 autoradiography

→ Two replication forks, representing the regions of active DNA synthesis

→ DNA 두 가닥이 동시에 복제됨: bidirectional replication

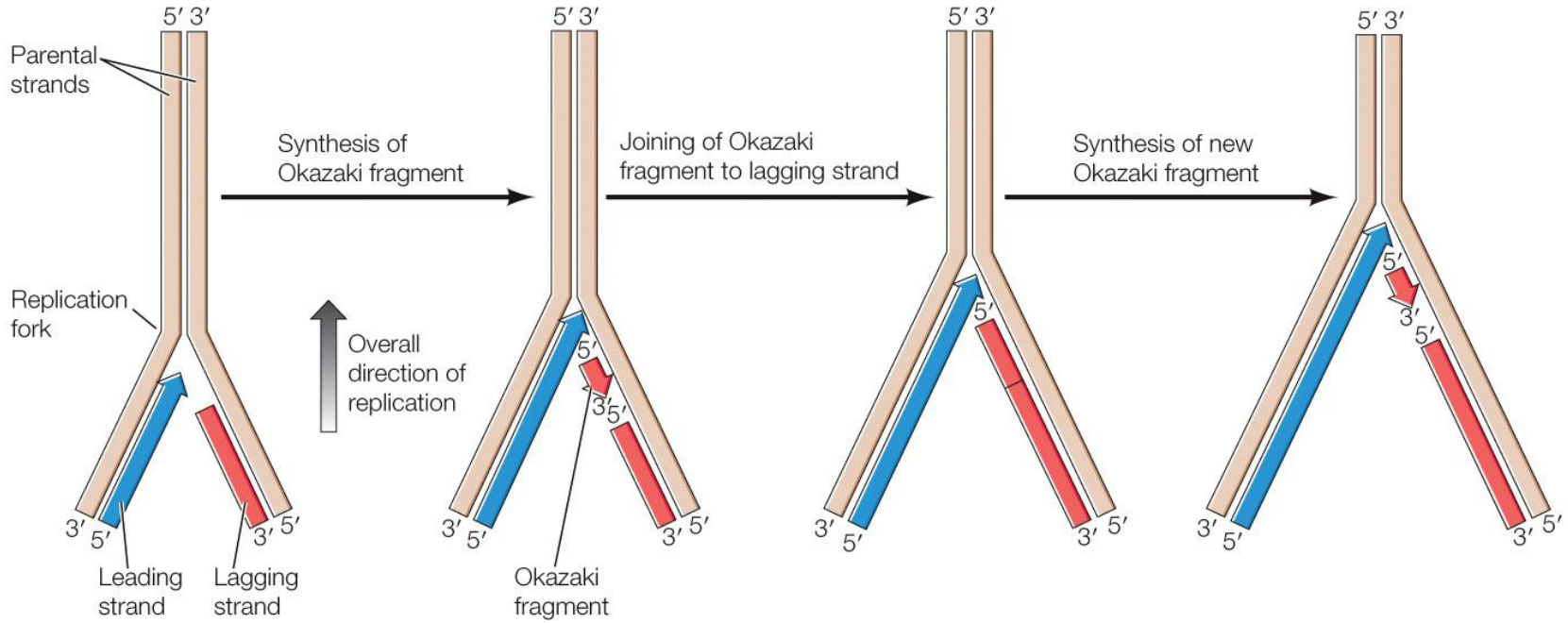


Replication of *E. coli* DNA

DNA 복제의 문제점과 해결방안

1. DNA structure; antiparallel
2. DNA synthesis; 5'→3' direction
3. DNA 두 가닥이 동시에 복제

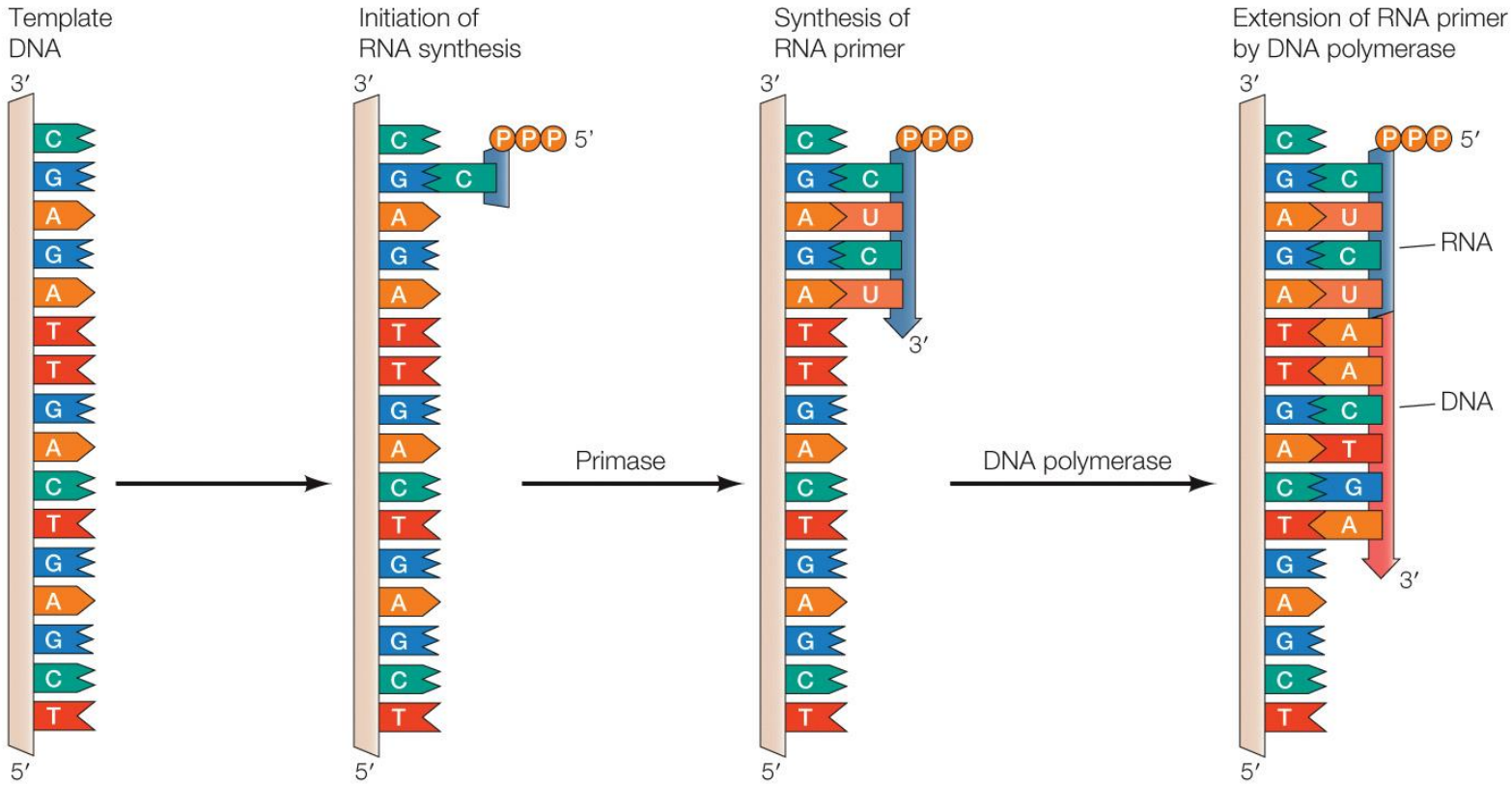
- ◆ Leading strand (선도가닥): continuous synthesis
- ◆ Lagging strand (지연가닥): discontinuous synthesis



Okazaki fragments are between 1,000 and 2,000 nucleotides long in *Escherichia coli* and are 150 nucleotides long in eukaryotes

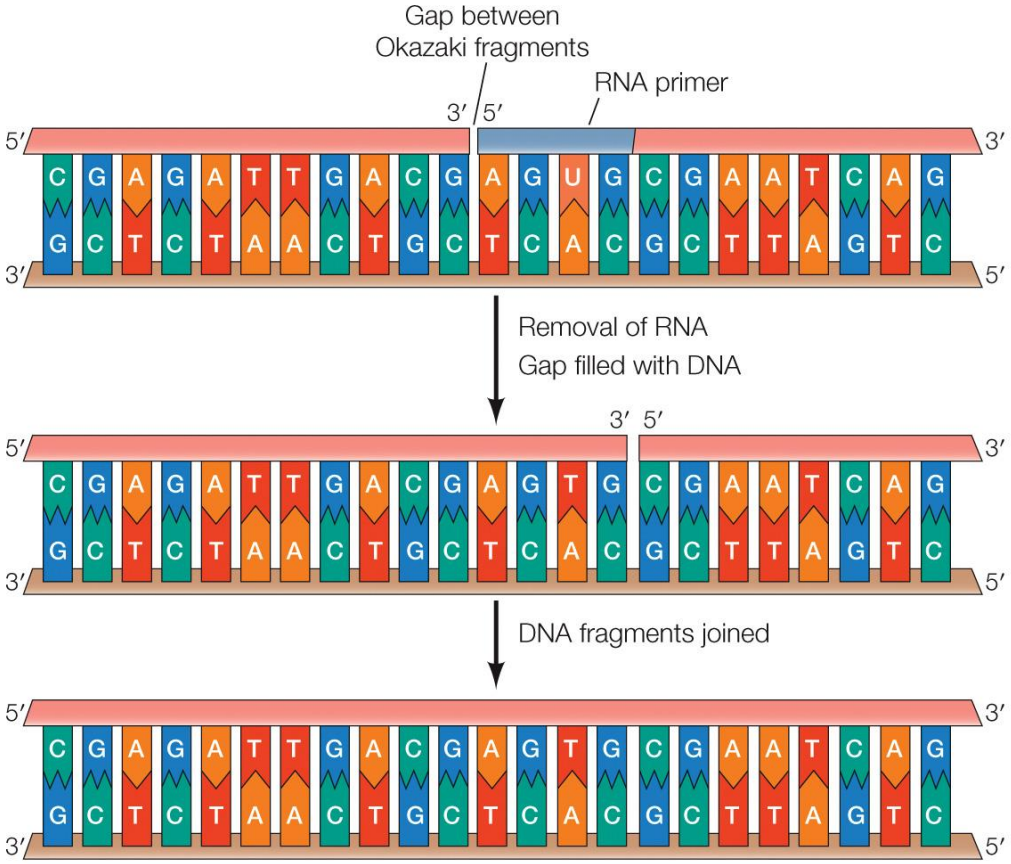
DNA polymerase의 primer 요구성

- Short fragments of RNA serve as primers for synthesis of Okazaki fragments.
- RNA synthesis can initiate *de novo.*
- **Primase** synthesizes short fragments of RNA that act as primers.

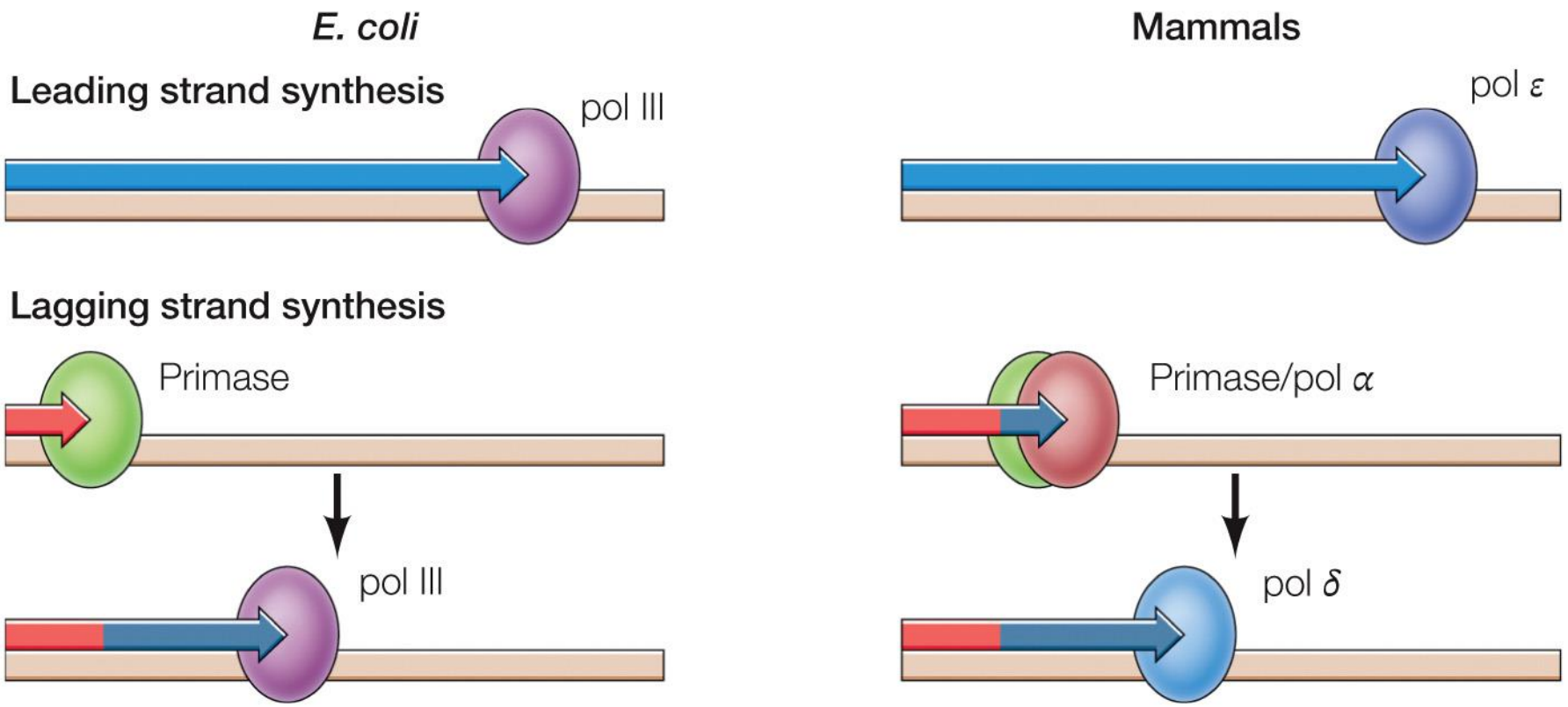


Removal of RNA Primers and Joining of Okazaki Fragments

- Prokaryotes; RNA primers are removed by polymerase I, an **exonuclease** that can hydrolyze RNA in either direction.
- Eukaryotes; RNA primers are removed by **RNase H** and 5′ to 3′ exonucleases. The resulting gaps are filled by polymerase *δ* and the fragments joined by DNA ligase.



Roles of DNA Polymerases in *E. coli* and Mammalian Cells



Polymerase Accessory Proteins

지속적인 DNA 복제

1. Clamp-loading protein

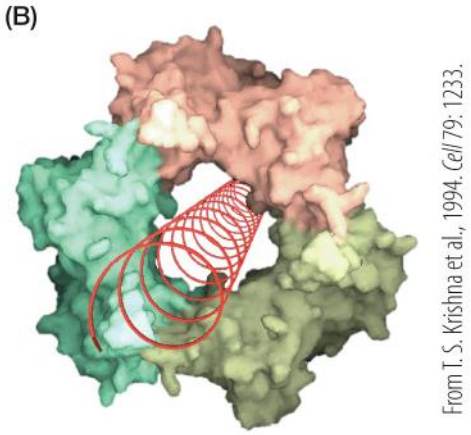
E. coli—γ complex

Eukaryotes– replication factor C (RFC)

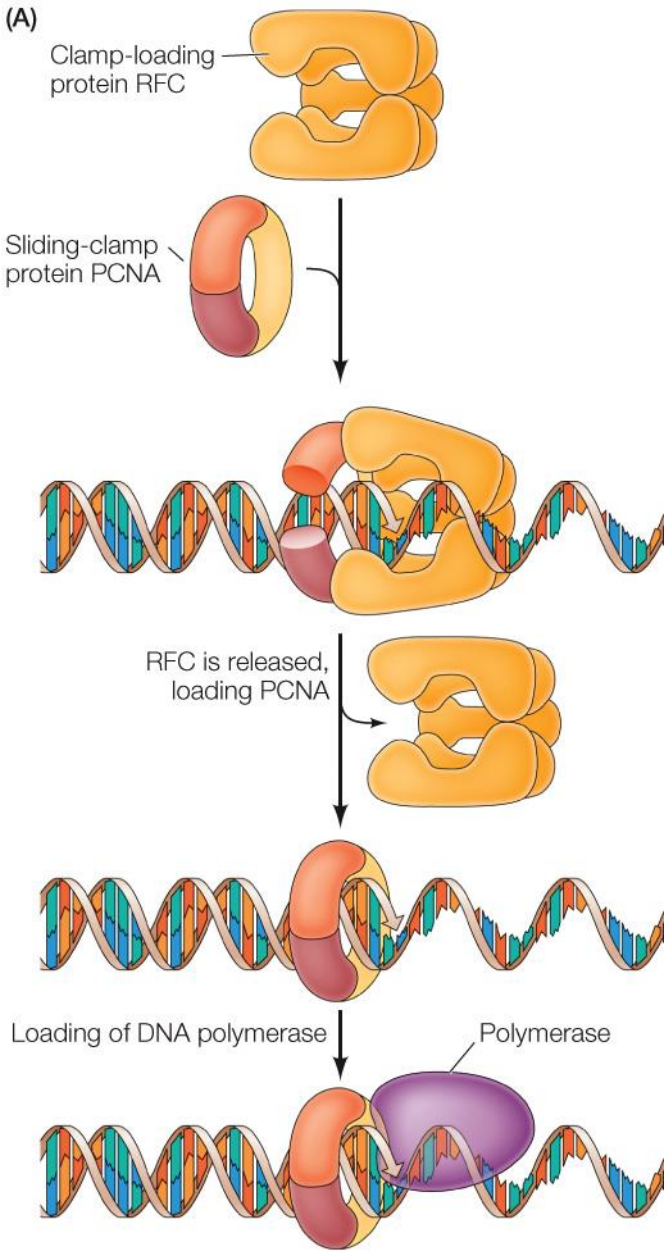
2. Sliding-clamp protein

E. coli—β protein

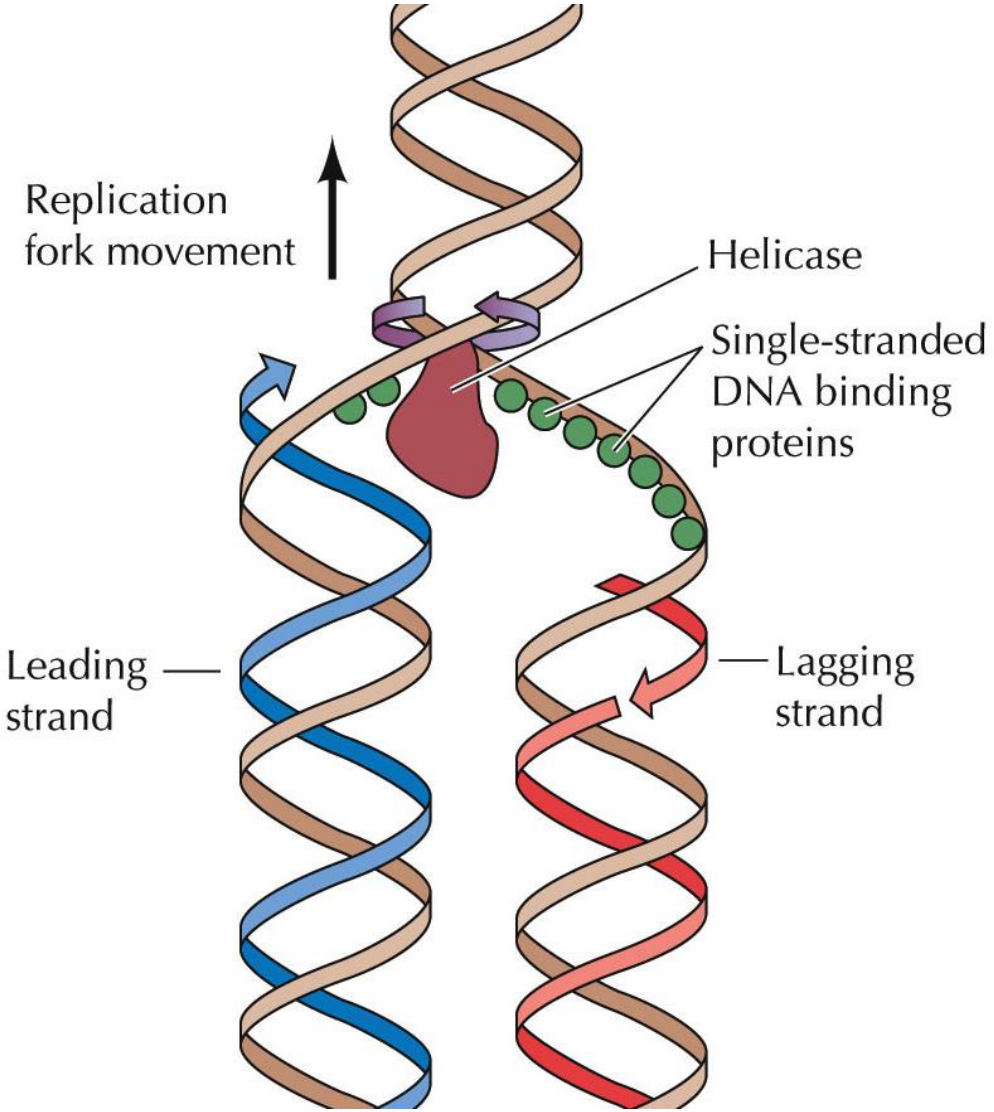
Eukaryotes– proliferating cell nuclear antigen (PCNA)



From T. S. Krishna et al., 1994. *Cell* 79: 1233.



Action of Helicases and Single-Stranded DNA-Binding Proteins

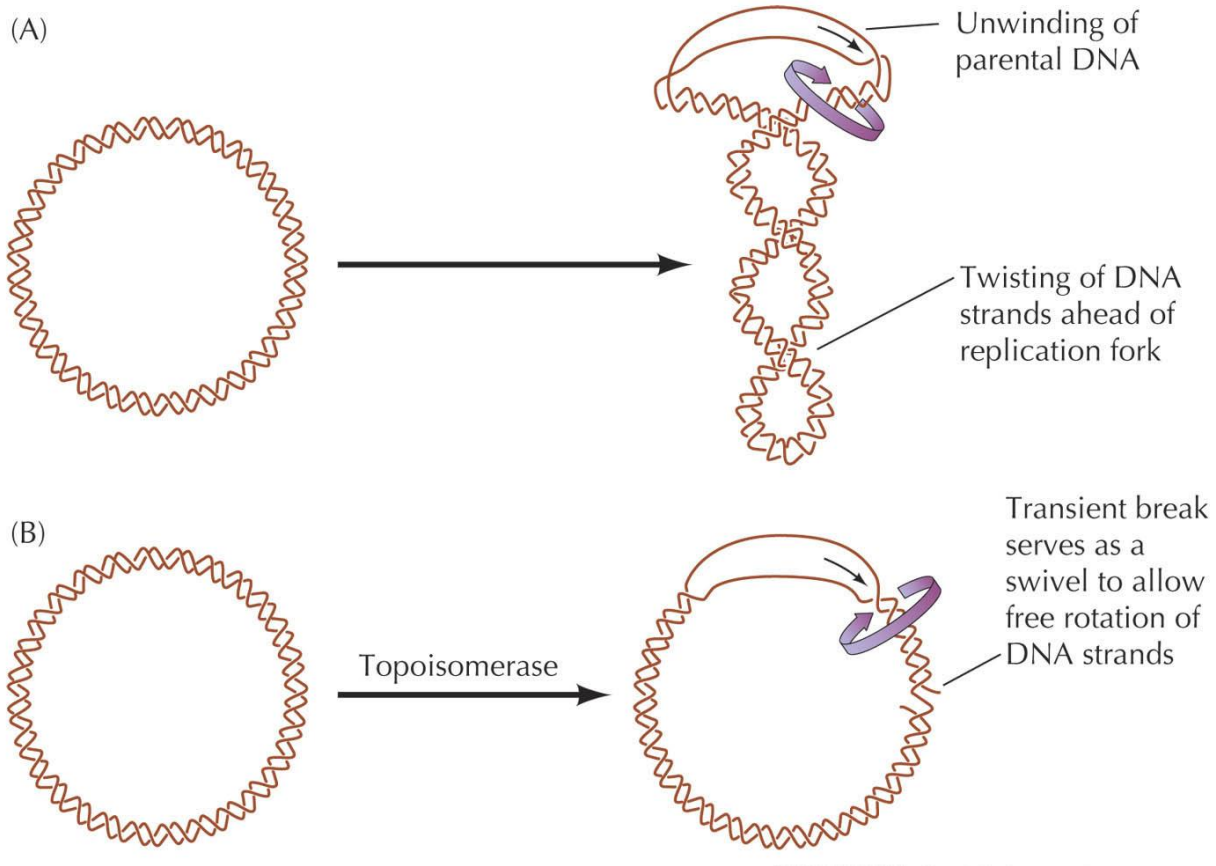


Action of Topoisomerases during DNA Replication

Unwinding of double stranded DNA

→ Rotation in a linear DNA, Twist in a circular DNA

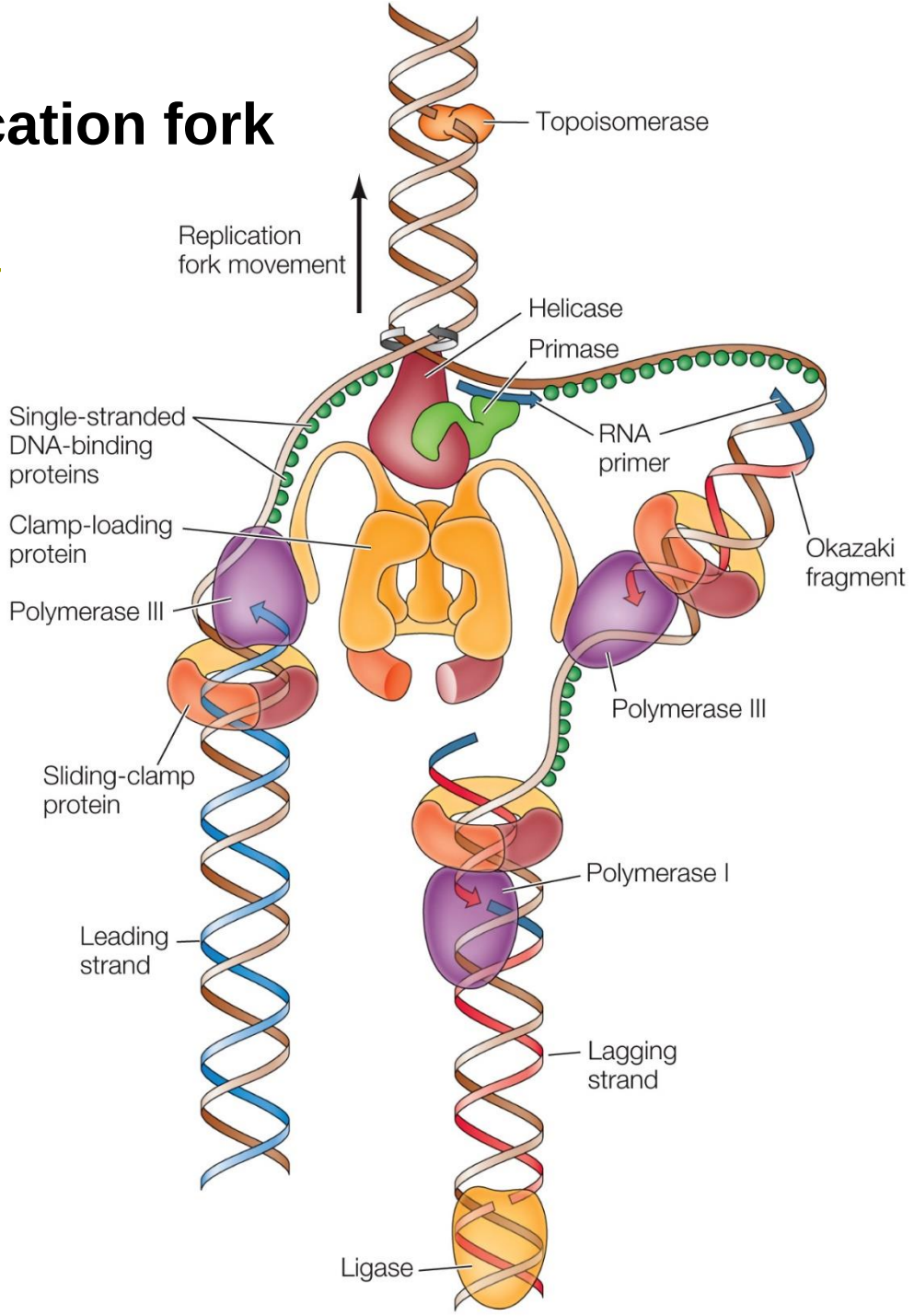
→Topoisomerases; reversible breakage and rejoining of DNA strands



Type I: 한가닥만 절단

Type II: 두가닥 동시에 절단
(복제DNA의 엉김을 해소)

Model of the *E. coli* replication fork



The enzymes involved in DNA replication act in a coordinated manner to synthesize both leading and lagging strands of DNA simultaneously.

The fidelity of DNA replication

DNA synthesis error rate

$1/10^3$; template-base pairing

$1/10^3$; Selection of the correct base for insertion by DNA pol

$1/10^3$; proofreading (교정능력)

$1/10^2$ ~10^3 ; Mismatch DNA repair

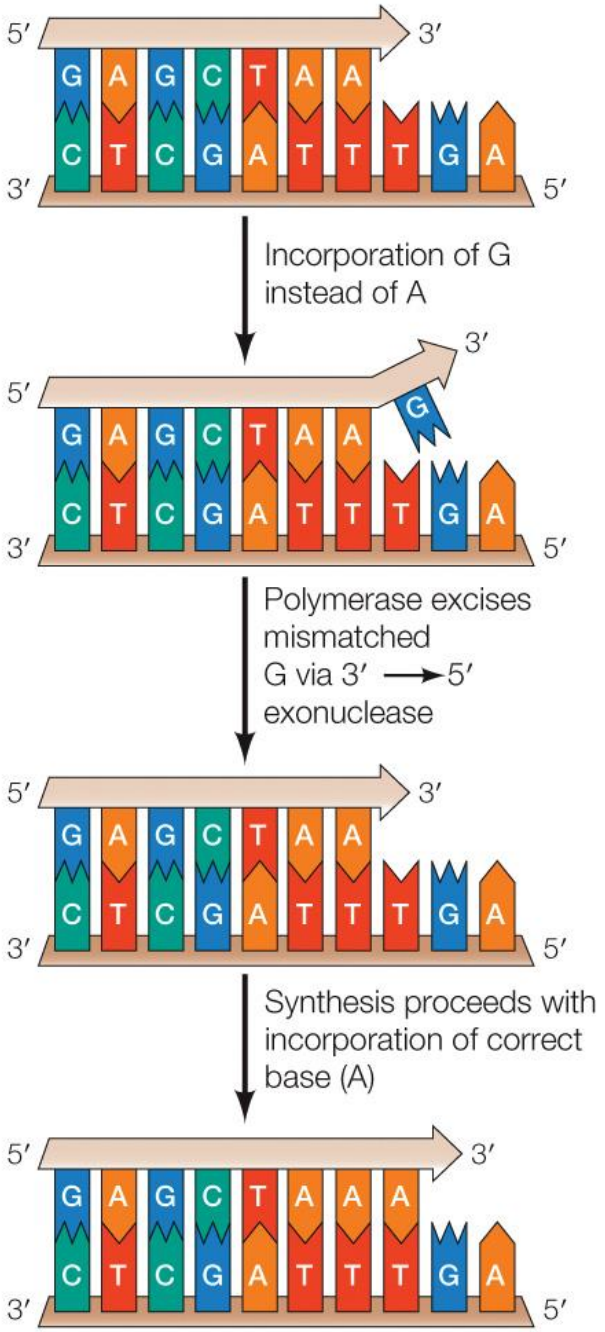
Proofreading

Mispaired nucleotide첨가시 이를 인식하여 다음

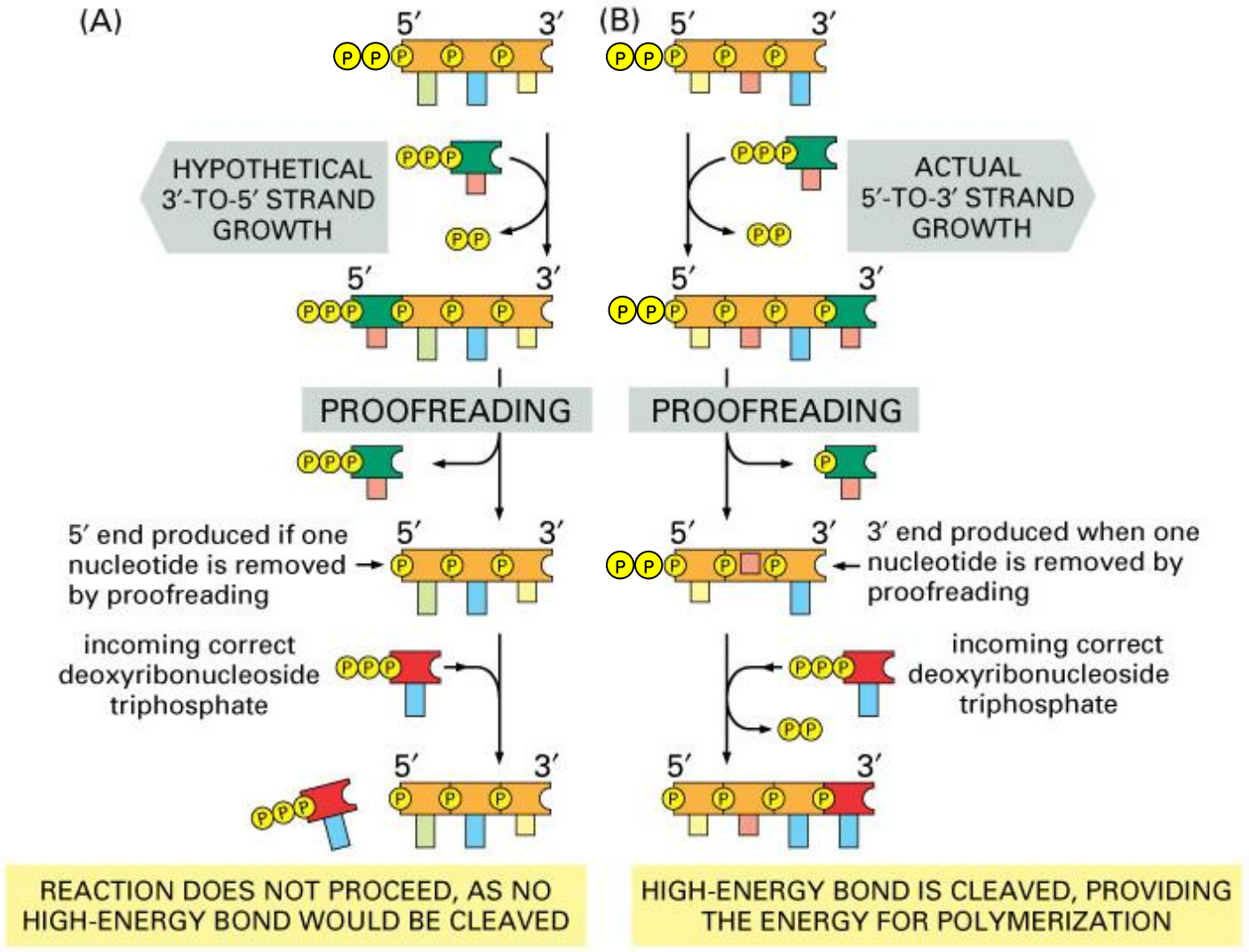
합성단계를 진행하지 않고, 잘못 첨가된 nucleotide를

3’→5’ exonuclease activity를 이용하여 제거하고

정확한 nucleotide로 대체하는 과정



Why DNA chains are synthesized only in the 5’ to 3’ direction ?



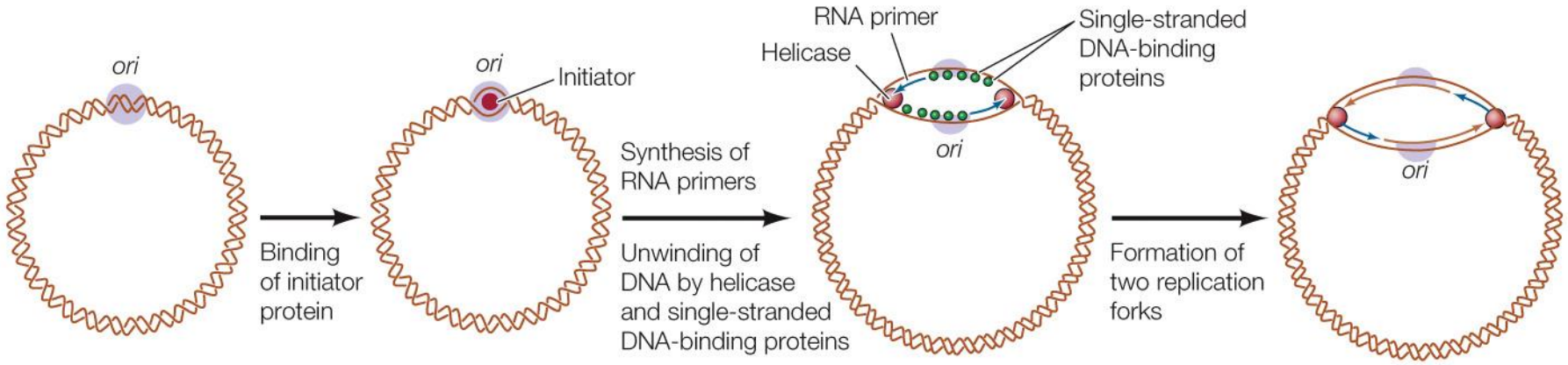
Origins and initiation of replication

Replication origin: 복제가 개시되는 지점

→binding sites for proteins that initiate the replication process (origin binding protein, OBP)

→Helicase를 끌어들여 이중가닥을 풀어 주형 DNA를 노출시킴

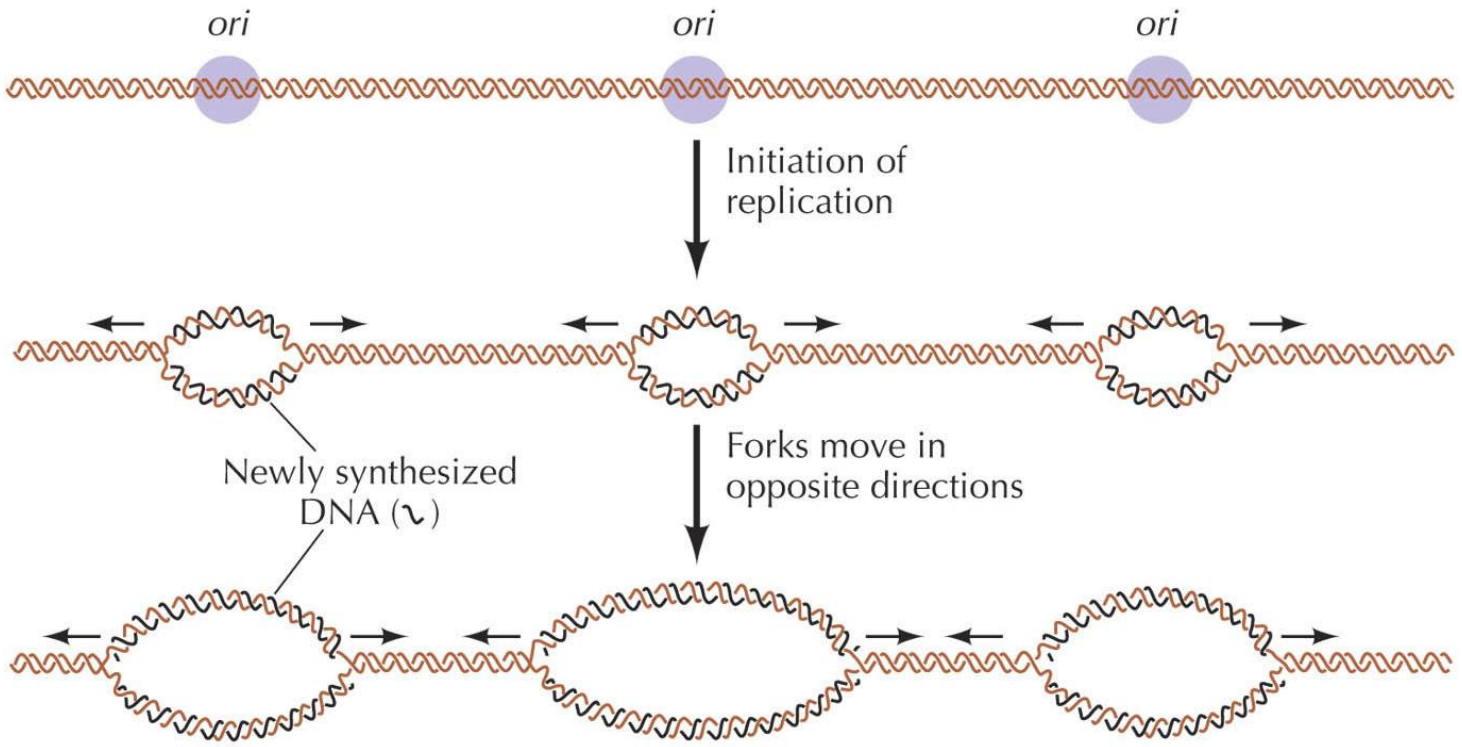
→ Single-stranded DNA-binding protein이 결합하고 primase에 의하여 RNA primer 생성



Origin of Replication in *E. coli*

Replication Origins in Eukaryotic Chromosomes

- E. Coli $4x10^6$→ single replication origins
- Human $3x10^9$, chromatin structure → multiple replication origins (인간 3만개)



Telomeres and telomerases: Replicating the ends of chromosomes

원핵세포--Circular DNA

진핵세포--linear DNA

→DNA polymerase는 5'→3'으로만 DNA를 합성

→진핵세포의 말단부분은 복제가 진행될수록 짧아짐

1985, Carol Greider & Elizabeth Blackburn

Telomerase는 자체의 RNA 주형을 이용하여 DNA를 합성하는 역전사 효소; Telomeric repeats 생성

→Primase 및 DNA pol에 의해 DNA 복제

→ Telomere (말단소립)는 단순반복서열을 가짐