Part III

Genetic information replication and flow

Chapter 16

DNA Biosynthesis and Recombination

The biological function of DNA

- Store genetic information
- Replicate genetic information
- Express genetic information
- Gene mutation

DNA Biosynthesis

DNA replication

-DNA synthesis while DNA as template

Reverse transcription

-DNA synthesis while RNA as template

Proofreading system

Correct polymerization errors and repair of damaged DNA

Section One

The general features of genome replication

How does DNA replication proceed?

1. **Does replication begin at random sites or at unique site?**

2. **Does DNA replication proceed in one direction or both directions?**

3. **The overall chain growth occurs in 5'→3', 3'→5', or bothdirections?**

The General Features of Genome Replication

1、semiconservative

2、bidirection

3、semidiscontinu

4、replication fork

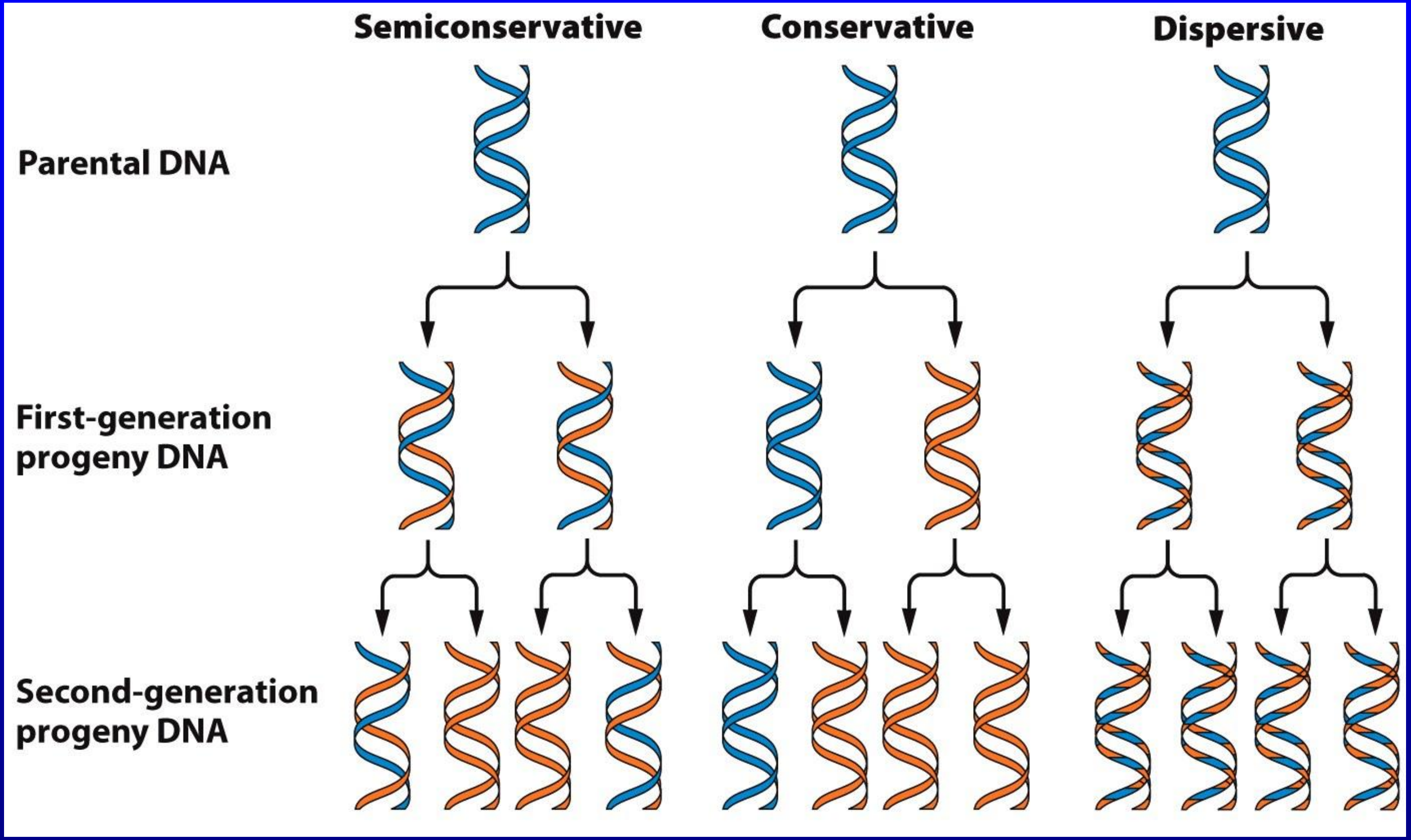
5、origin contains short repeat sequences

6、needs priming

7、multi-enzymes and protein participate

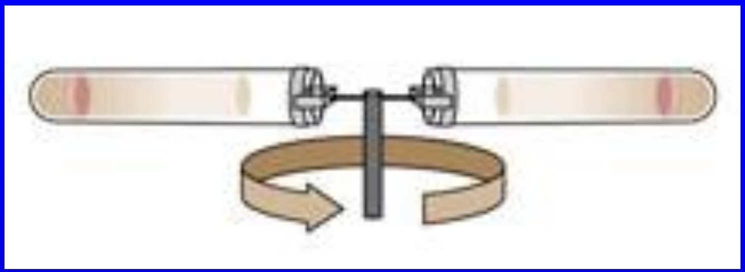
8、high fidelity

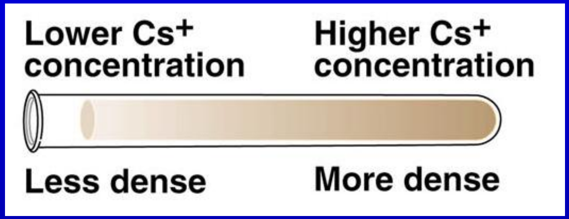
1. DNA Replication is Semiconservative



Possible Models of DNA Replication

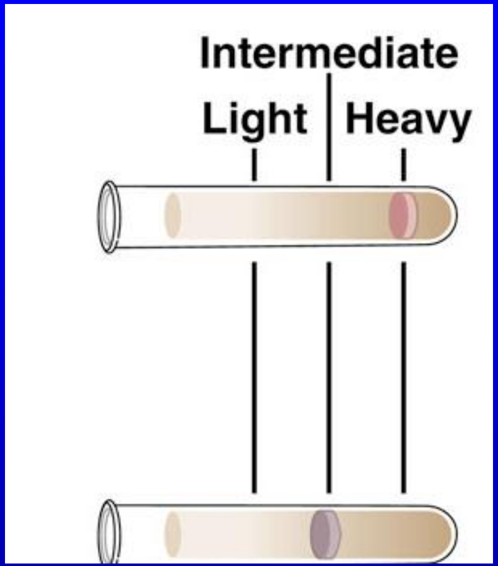
CsCl Density Gradient Centrifugation





DNA

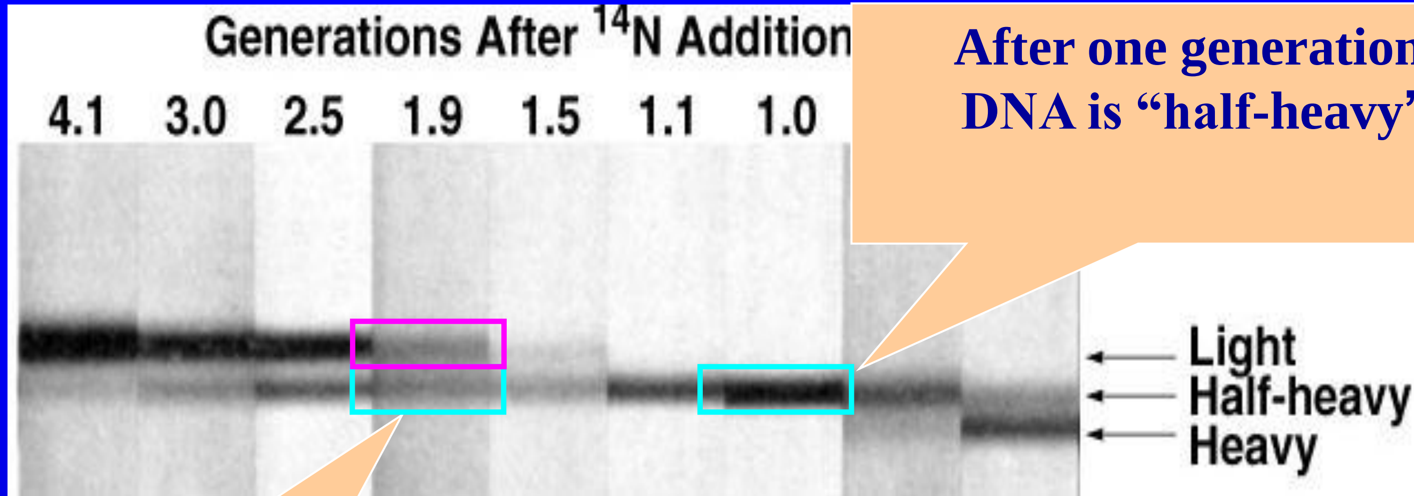
^{14}N



^{15}N

Matthew Meselson and Franklin Stahl experiment in 1958

- **Grow *E. coli* in the presence of ^{15}N (a heavy isotope of Nitrogen) for many generations。Cells get heavy-labeled DNA**
- **Switch to medium containing only ^{14}N (a light isotope of Nitrogen)**
- **Collect sample of cells after various times**
- **Analyze the density of the DNA by centrifugation using a CsCl gradient**



After one generation, DNA is "half-heavy"

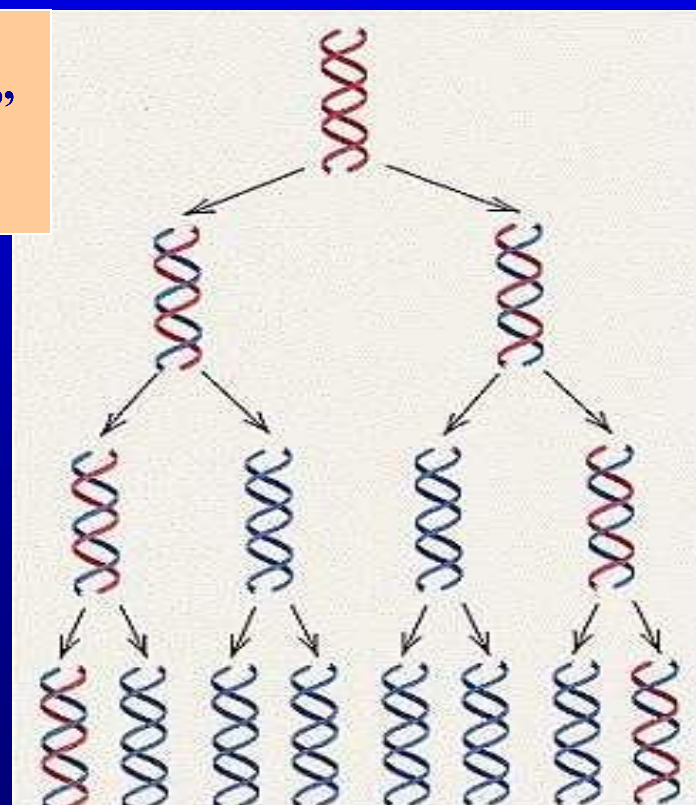
Gener n

After ~ two generations, DNA is of two types: "light" and "half-heavy"

1

2

3



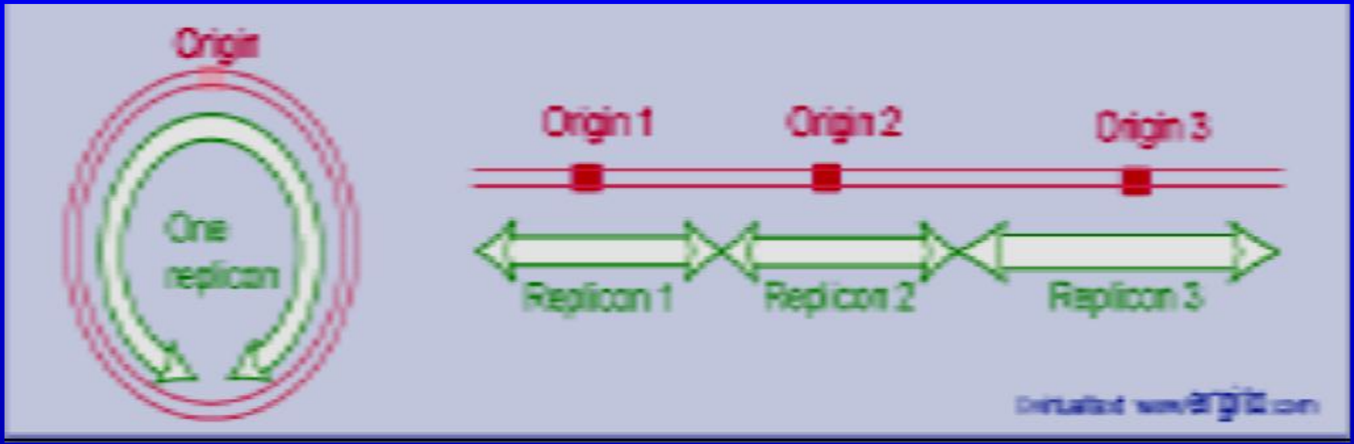
Replicon (复制子）

Replicon : The unit of DNA in which an individual act of replication occurs is called the replicon.

(A unit of the genome in which DNA contain a region from origin to terminator)

Each replicon "fires" once and only once in each cell cycle.

Replicon organization differs in prokaryotes and eukaryotes



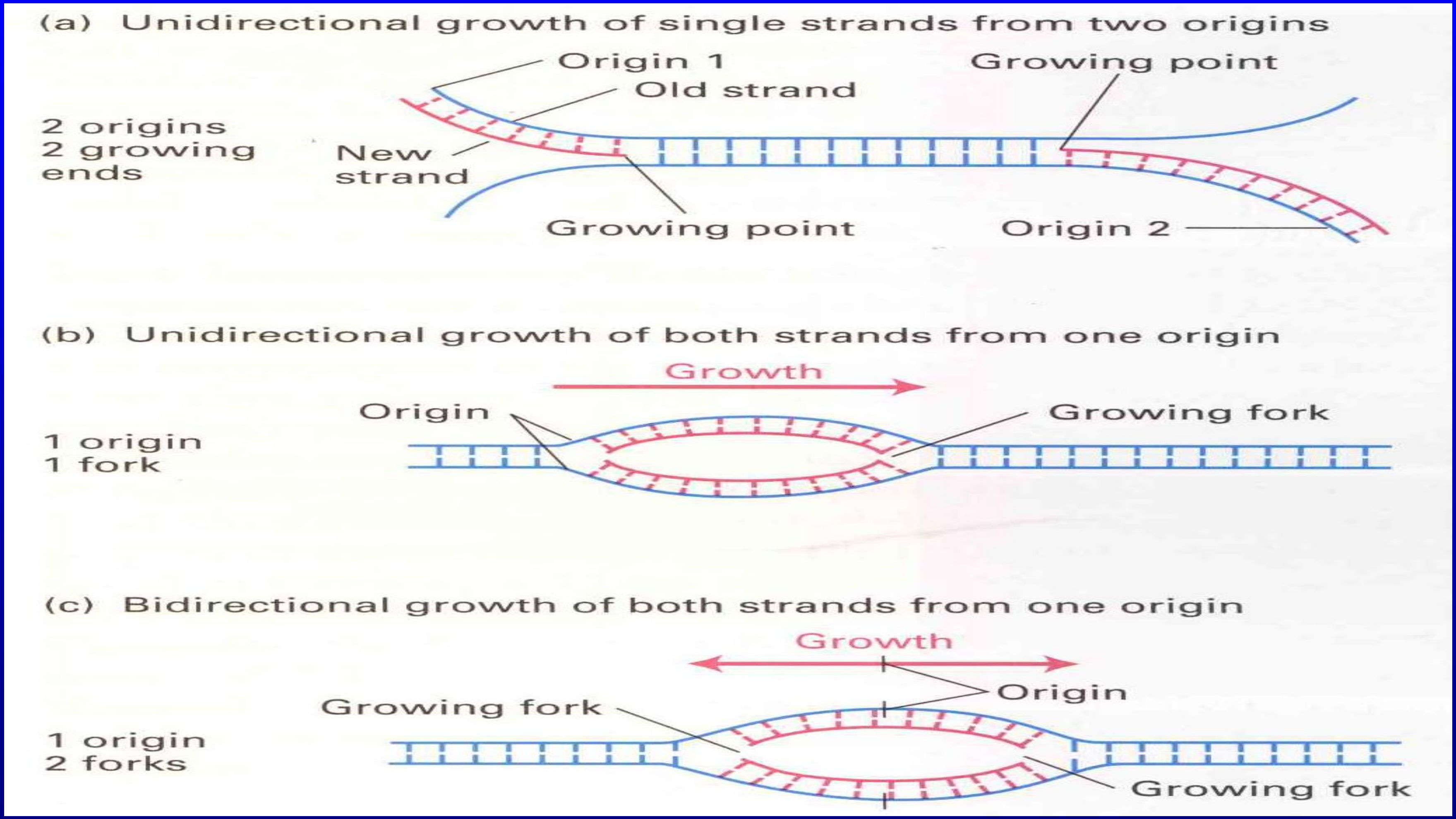
A bacterium usually has a circular chromosome that is replicated from a single origin, but a eukaryotic chromosome has many origins, each defining a separate replicon.

The speed of replication

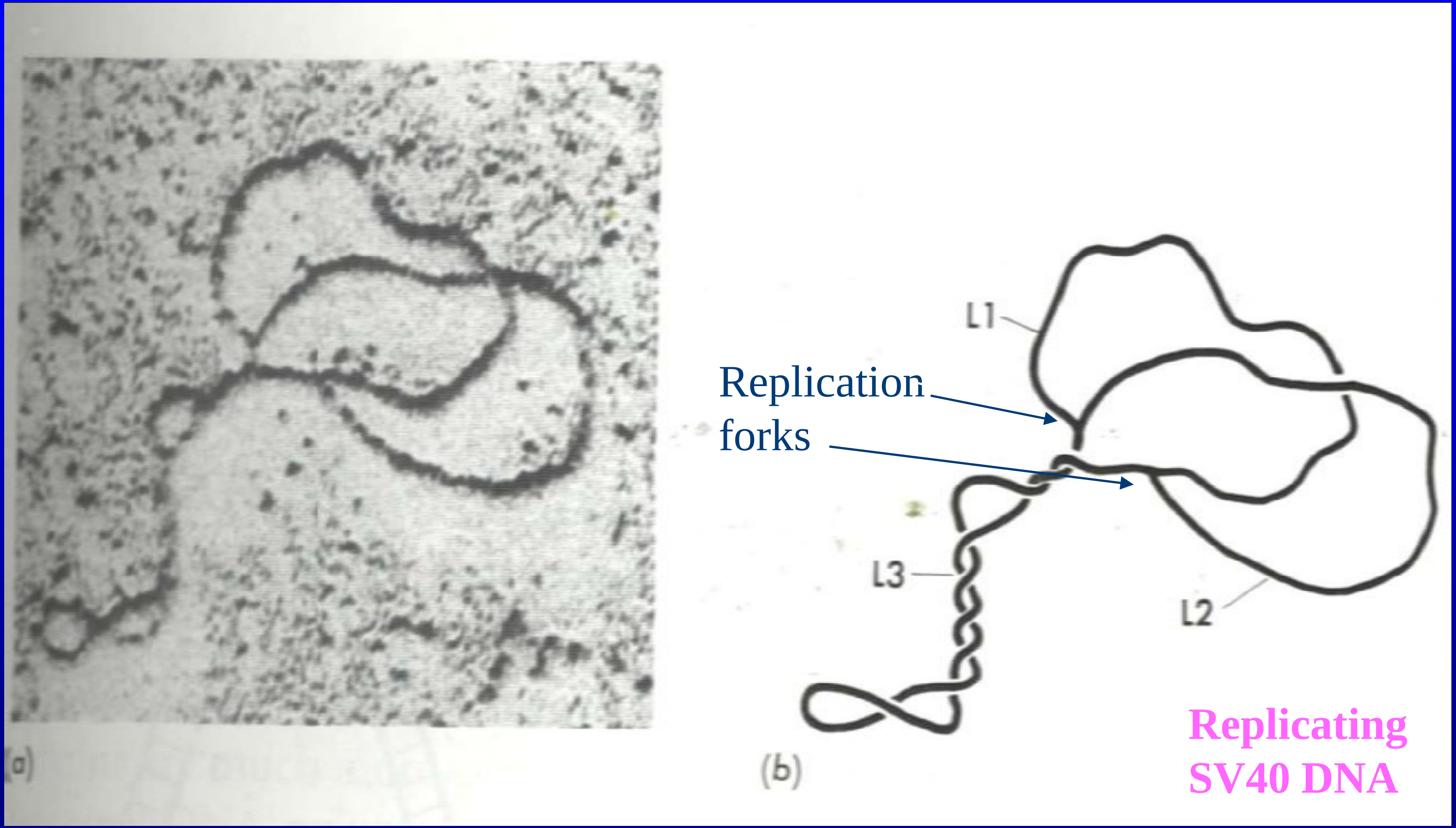
Organism	Replicons	Length	Movement
Bacterium	1	4200 kb	50,000 bp/min
Yeast	500	40 kb	3,600 bp/min
Fruit fly	3,500	40 kb	2,600 bp/min
Toad	15,000	200 kb	500 bp/min
Mouse	25,000	150 kb	2,200 bp/min
Plant	35,000	300 kb	

- ✓ **A chromosome is divided into many replicons.**
- ✓ **Eukaryotic replicons are 40-100 kb in length**
- ✓ **Individual replicons are activated at characteristic times during S phase**

2、Replication is bidirectional



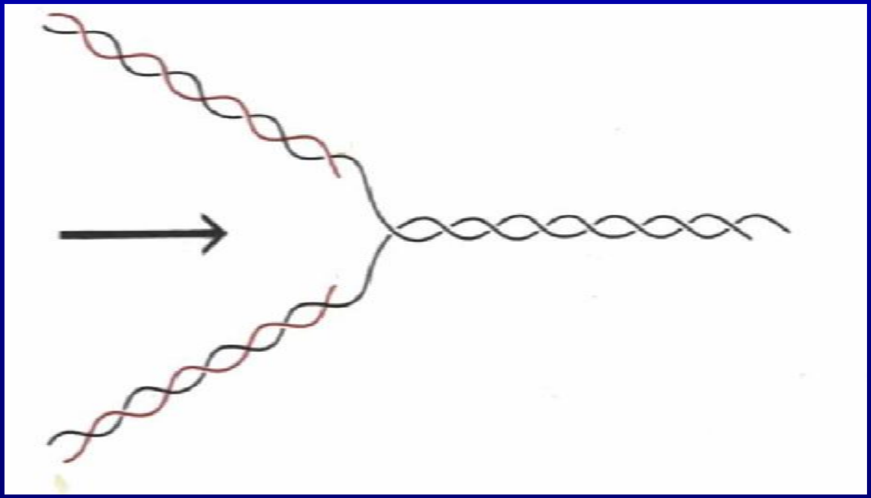
Evidence points to bidirectional replication



3、Replication forks

Points at which separation of the strands and synthesis of new DNA takes place is known as the replication fork.

The replication fork is Y-shaped. Two arms (V) are separated strands which act as the template and DNA synthesis is actively taking place. The body (I) is the parental DNA.

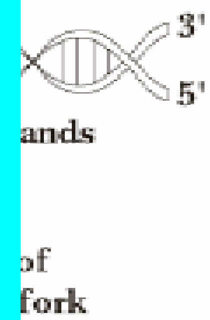


4、DNA replication is semi-discontinuous

- Reiji Okazaki discovered (in 1968) that a significant proportion of newly synthesized DNA exists as small fragments!

- The length of Okazaki fragments is about 1000-2000 bp, but shorter in Eukaryotes (100-200 bp).

- These so-called Okazaki fragments are joined by DNA ligases to form one of the daughter strands;



-Continuous additions of nucleotides to 3' end

Strand synthesized continuously – leading strand

Strand growing away from fork

-Synthesized discontinuously As fragments

-Before synthesis the fork must move away

-Once initiated the fragment grows 5' to 3'

-Subsequently each fragment is linked to the next

Strand synthesized discontinuously – lagging strand

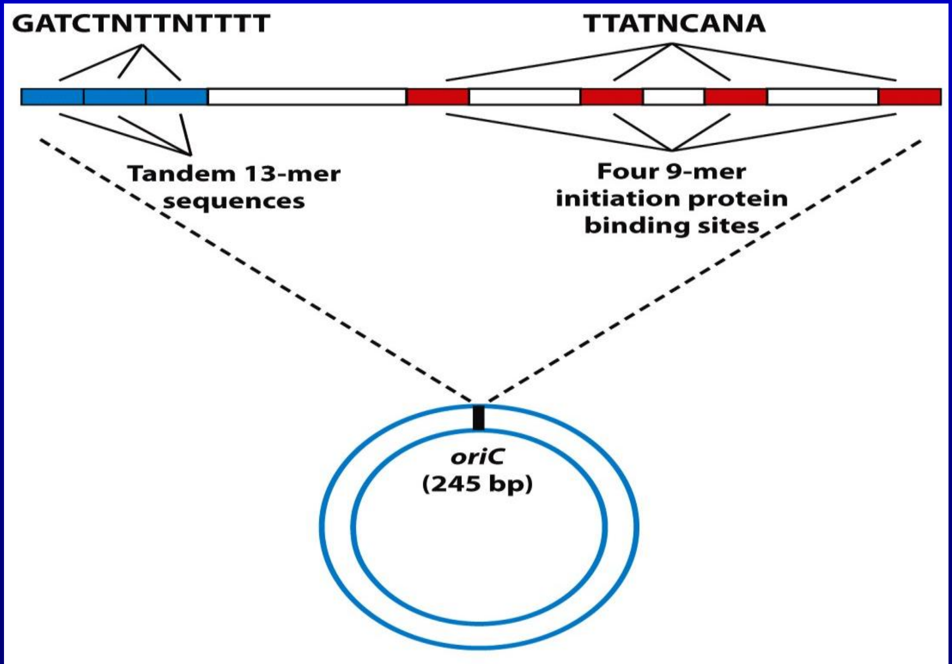
SUMMARY: Features of DNA Replication

- DNA replication is semiconservativ
  - Each strand of template DNA is being copied.
- DNA replication is bidirectional
  - Bidirectional replication involves two replication forks, which move in opposite directions
- DNA replication is semidiscontinuous
  - The leading strand copies continuously
  - The lagging strand copies in segments (Okazaki fragments) which must be joined

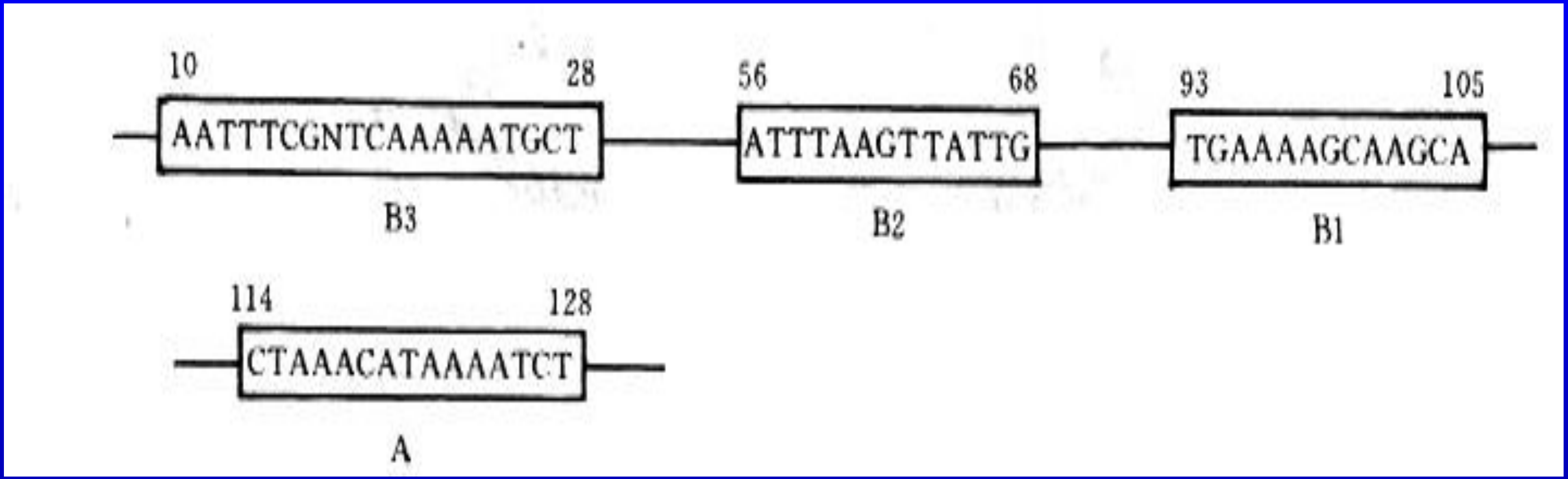
5、Origin of Replication contains short repeat sequences

- The origin of replication in *E. coli* is termed ***oriC***
  - **ori**gin of **C**hromosomal replication

- Important DNA sequences in *oriC*
  - **AT-rich region**
  - **DnaA boxes**

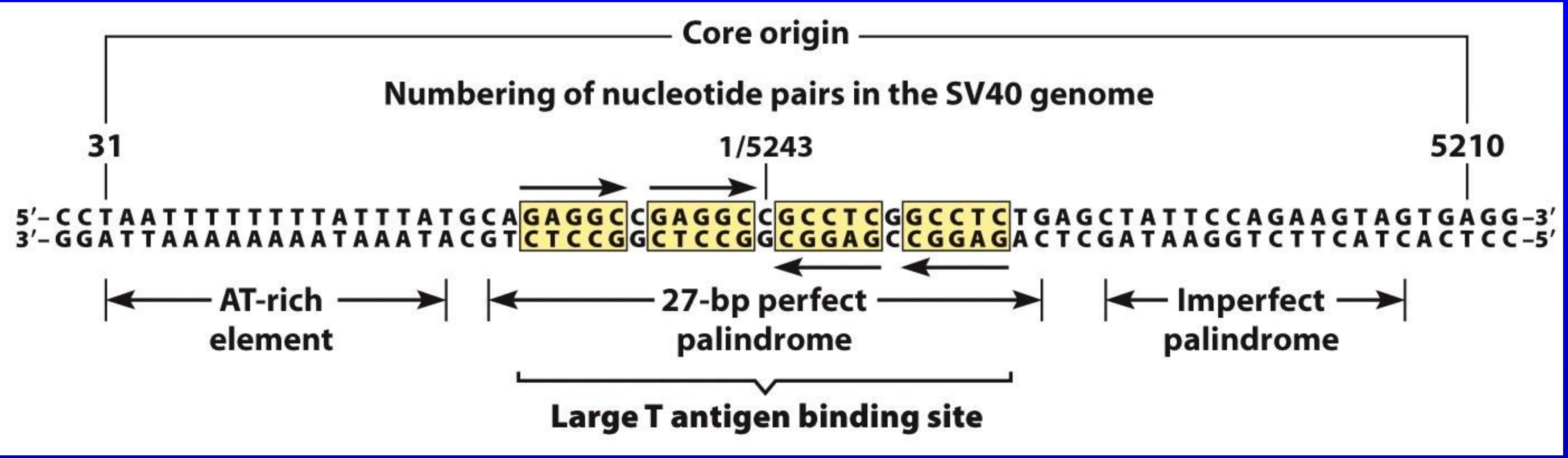


Replication origins isolated in yeast



- **ARS (autonomous replication sequence) is an origin for replication in yeast. The common feature among different *ARS* sequences is a conserved 11 bp sequence called the A-domain.**
- **An *ARS* extends for ~50 bp and includes a consensus sequence (A) and additional elements (B1-B3).**

The Core Origin of Replication in SV 40



Palindrome：反向互补序列，回文序列

Was it a car or a cat I saw

客上天然居，居然天上客

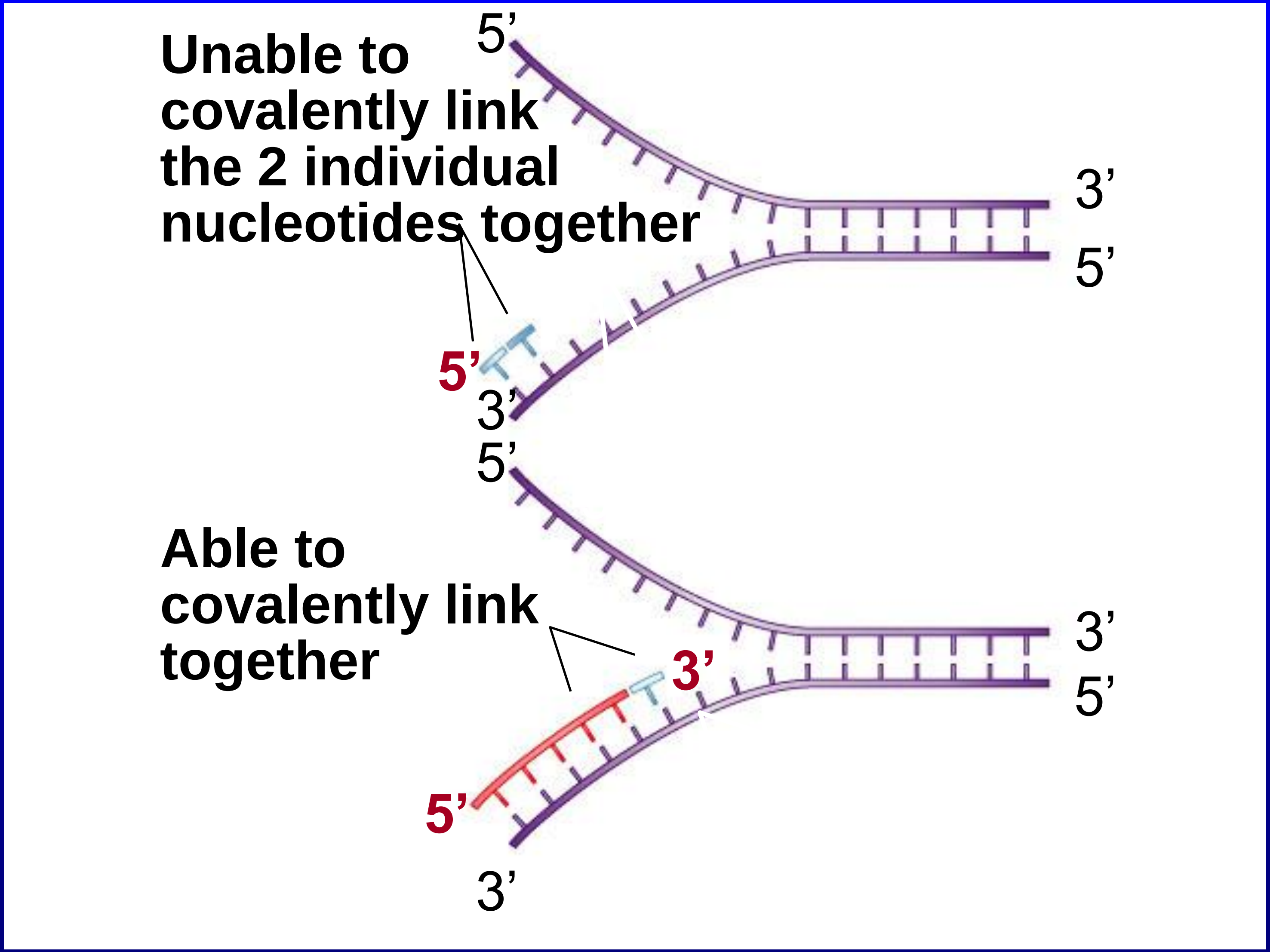
General features of replication origins：

1、several short repeat sequences；

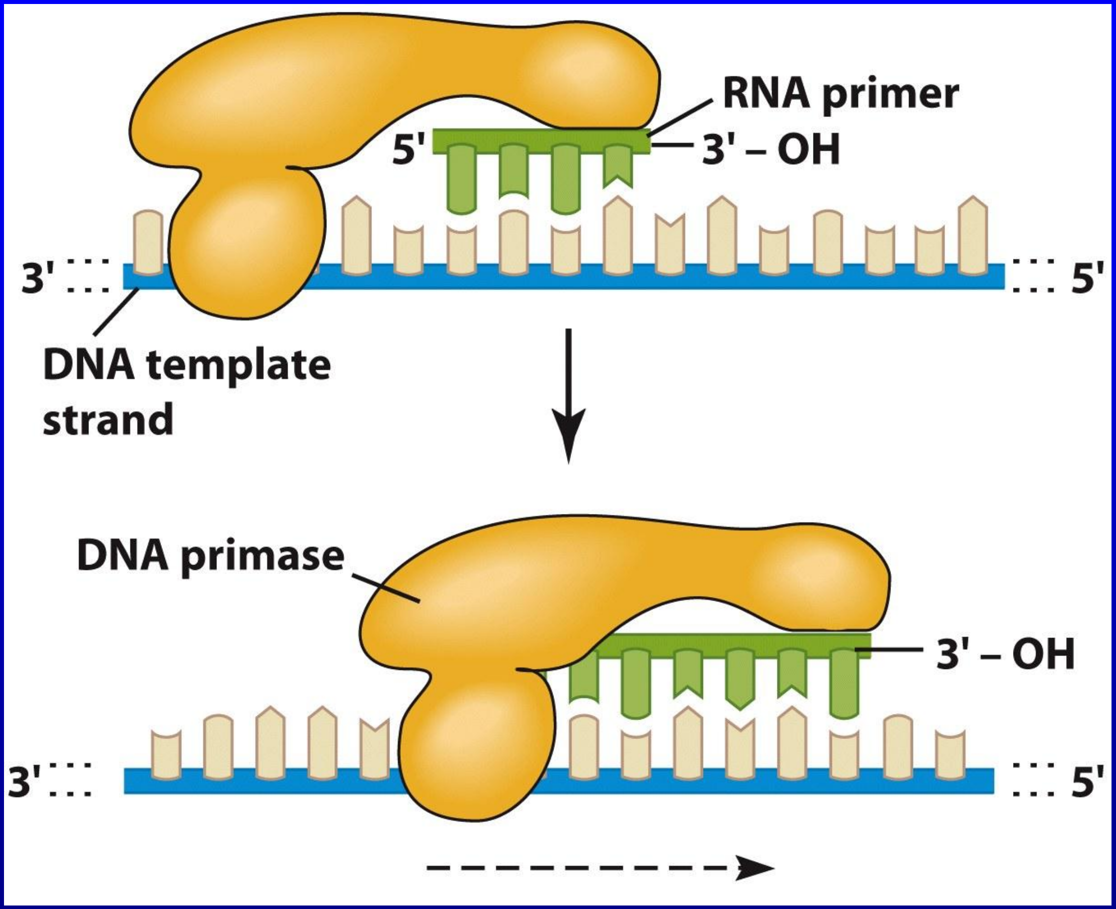
2、binding with repliction initiation protein

3、A/T rich sequences。

6、DNA replication needs priming



Most DNA replications are primed by RNA



- ➢The primase is a RNA polymerase different with that in the transcription.
- ➢The primer is a fragment of RNA about 10-20bp approximately

There are also DNA priming or nucleotide priming

Figure 12.16 The rolling circle generates a multimeric single-stranded tail.

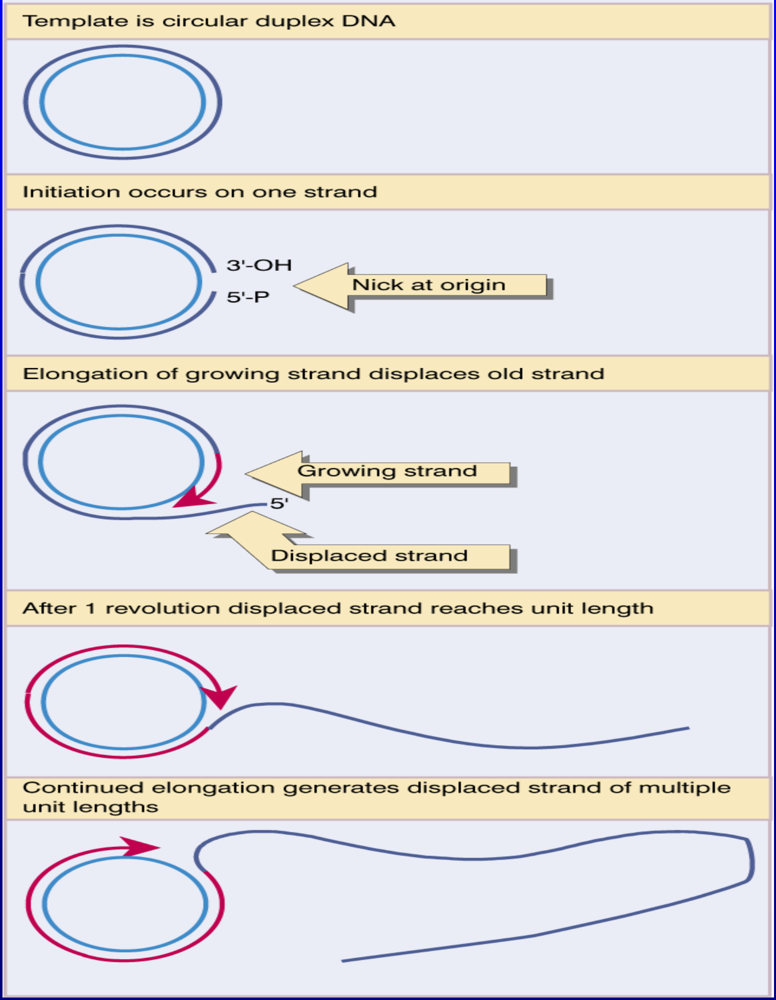
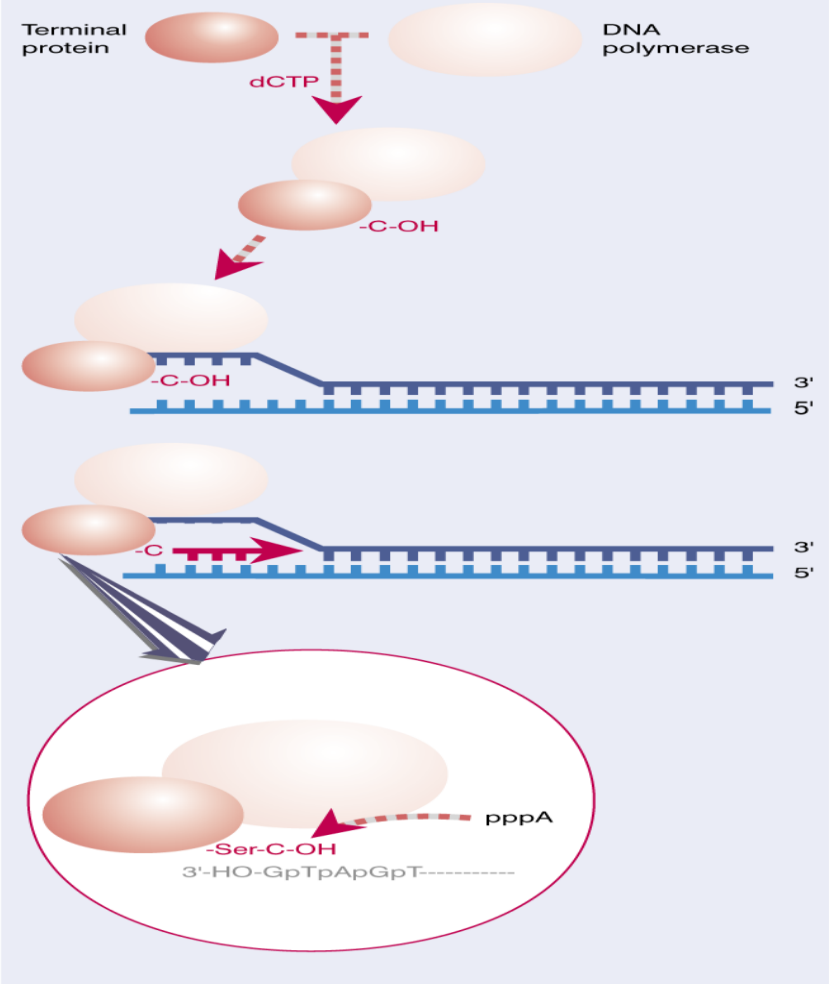


Figure 12.15 Adenovirus terminal protein binds to the 5' end of DNA and provides a C-OH end to prime synthesis of a new DNA strand.

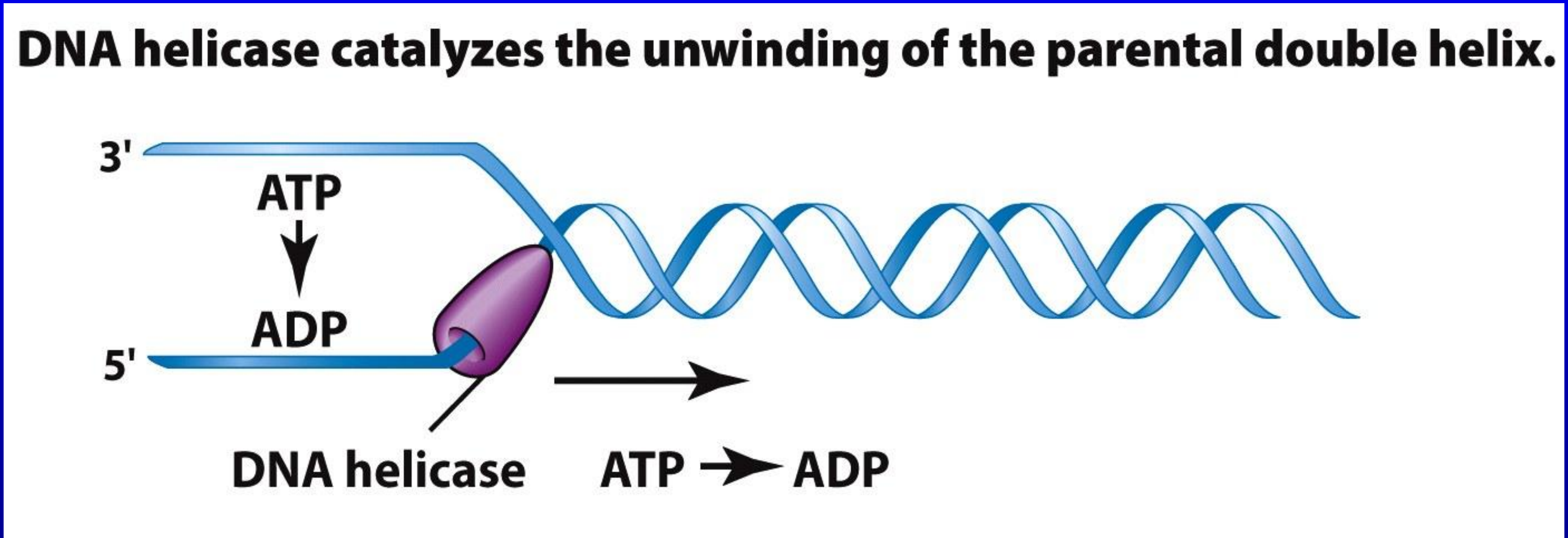


7、Multi-enzymes and proteins participate in DNA replication

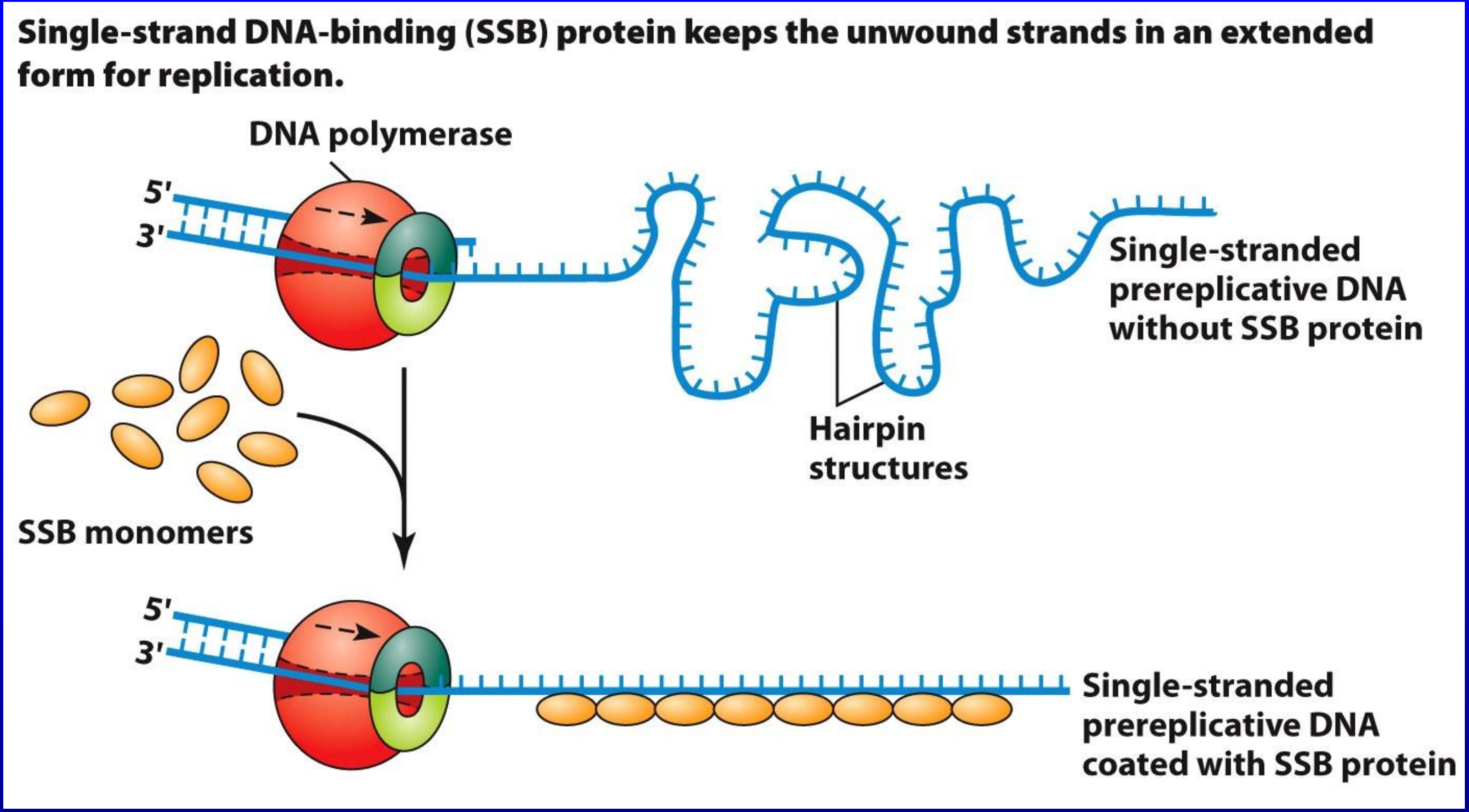
1 DNA dependent DNA polymerase
2 primase
3 ligase
4 helicase, gyrase
5 single strand binding protein
6 topoisomerase

Birth place of Taq： yellow stone

DNA Helicase Unwinds the Parental Double Helix

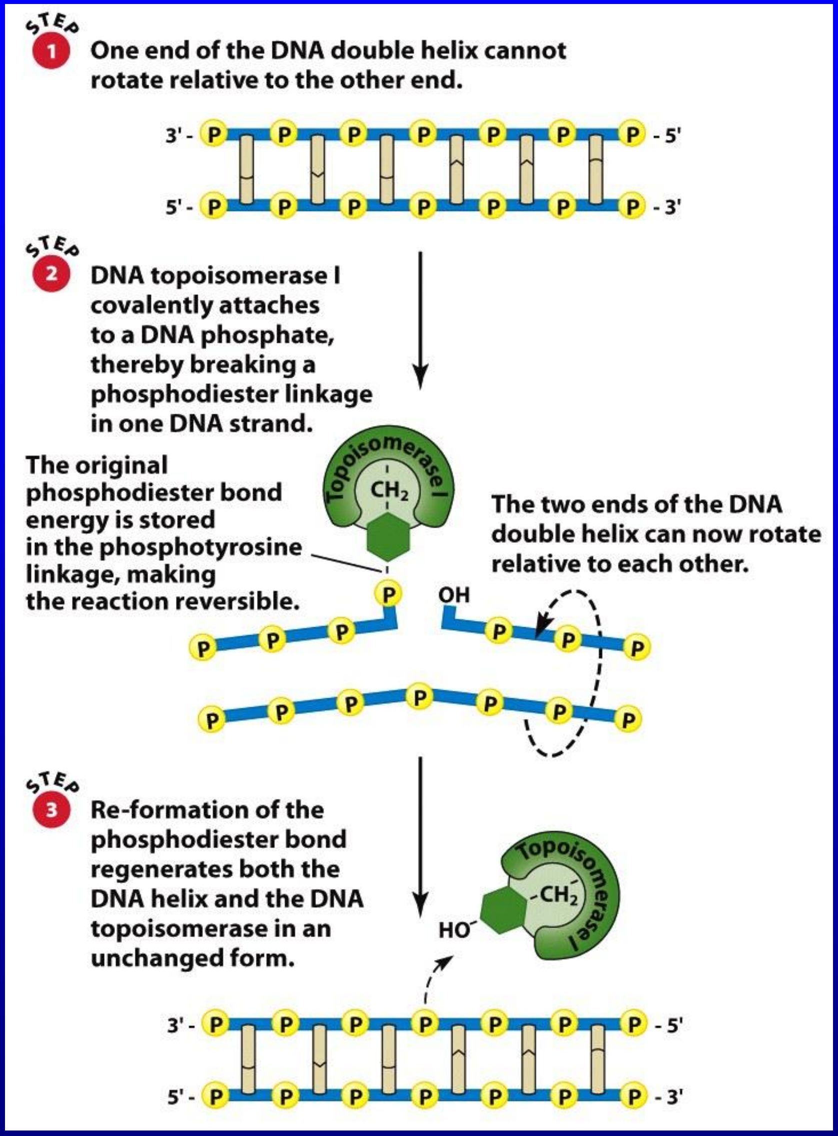


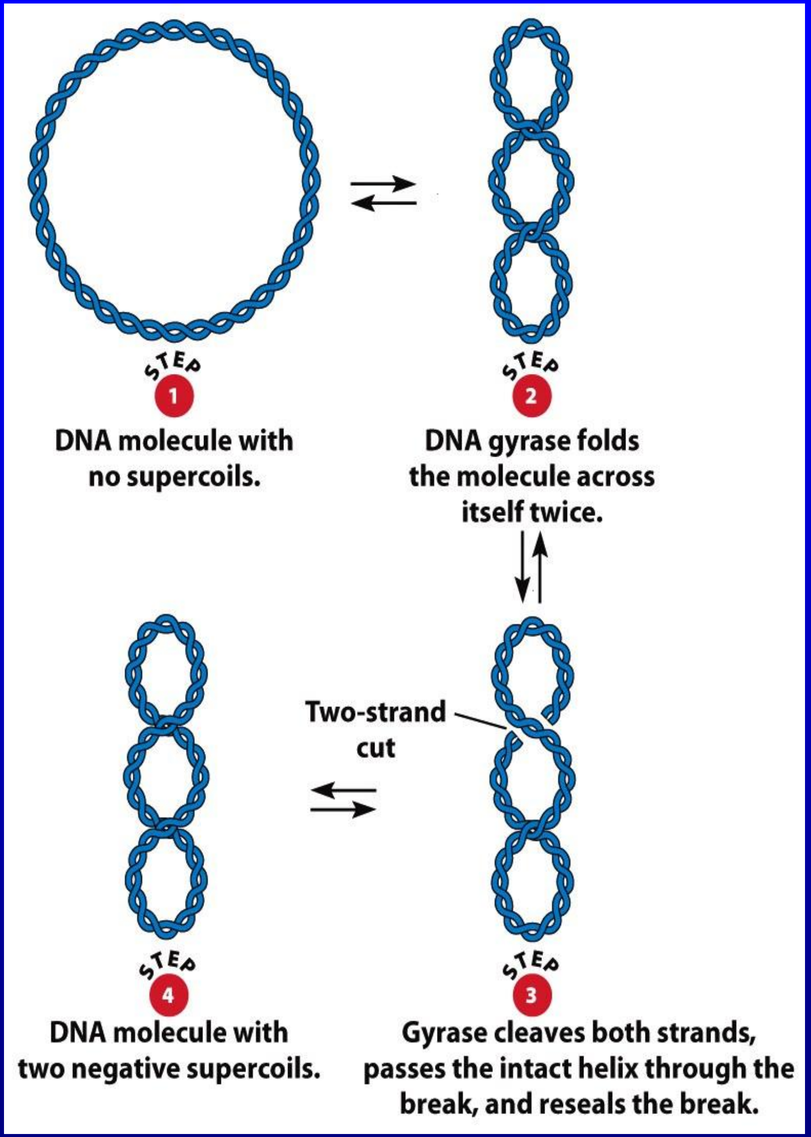
Single-Strand DNA Binding (SSB) Protein



Replication is 100 times faster when these proteins are attached to the single-stranded DNA.

DNA Topoisomerase

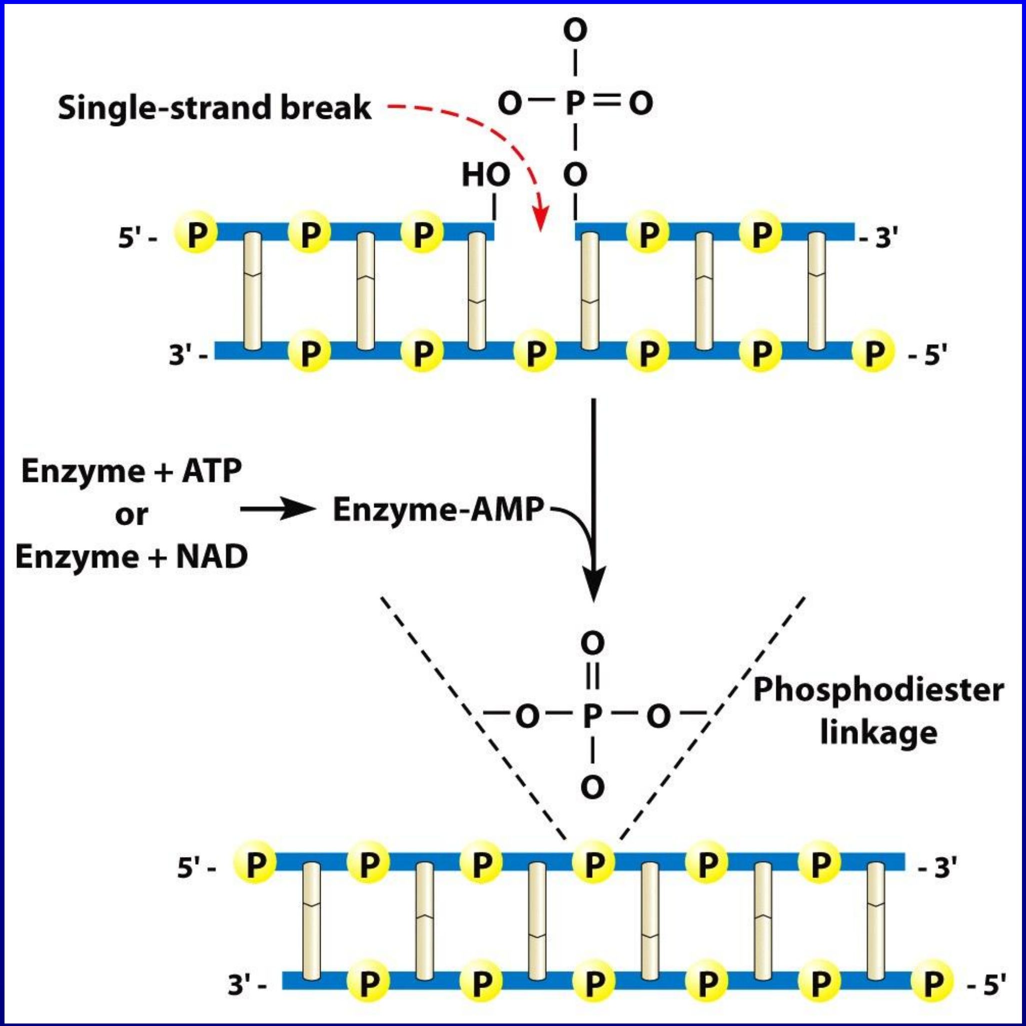




I Produces Single-Strand Breaks in DNA

II Produces Double-Strand Breaks in DNA

DNA Ligase Covalently Closes Nicks in DNA



8、High Fidelity of DNA Replication

- Base pair system： 10^{-4} ~ 10^{-5}
- DNA Polymerase III and δ : ~10^{-7}

  Presynthetic error control (合成前误差控)

  Proofreading control (校正控制)
- DNA repair system

Features of DNA polymerases

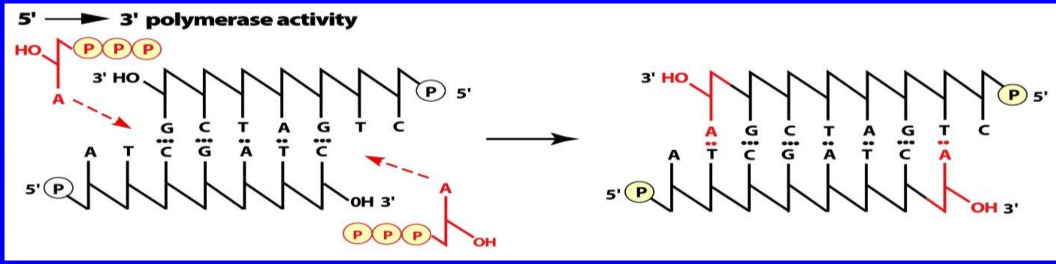
- DNA as templet
- Substrate are dNTP
- Require a free 3'-OH at the end of a primer
- Form Phosphodiester bond
- Base pair principle
- Direction 5' to 3'
- Exonuclease activity

DNA Polymerases in E.coli

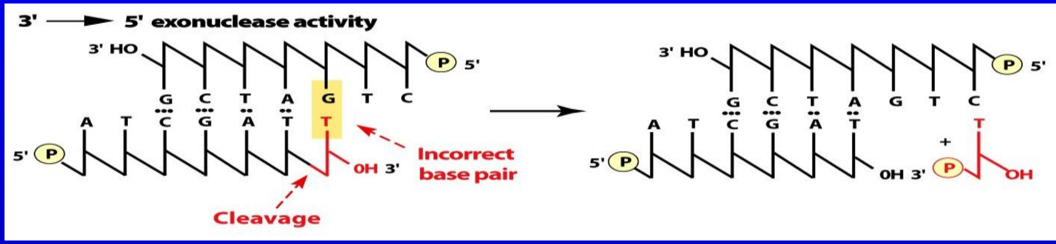
I	major repair enzyme
II	major repair enzyme
III	replicase
IV	SOS repair
V	SOS repair

DNA Polymerase I

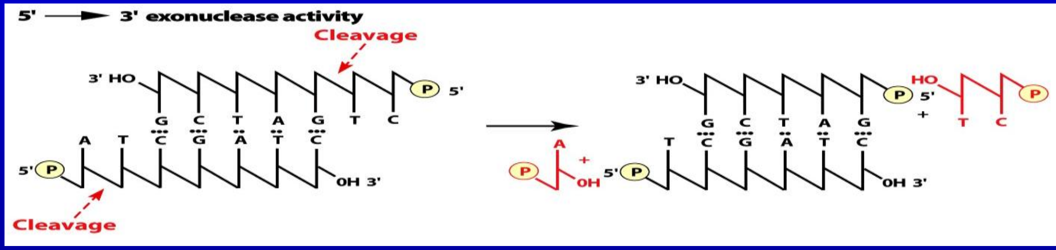
5′➔3′ Polymerase Activity

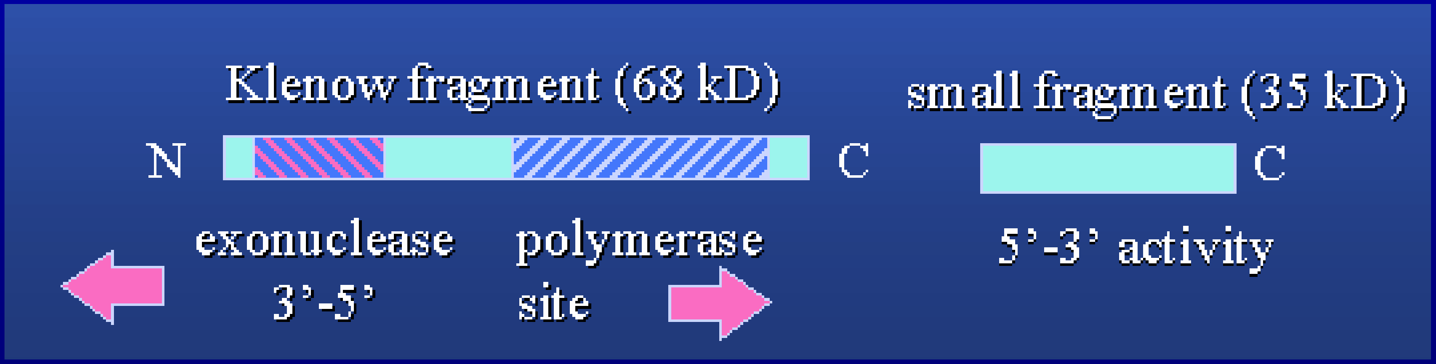


3′➔5′ Exonuclease Activity

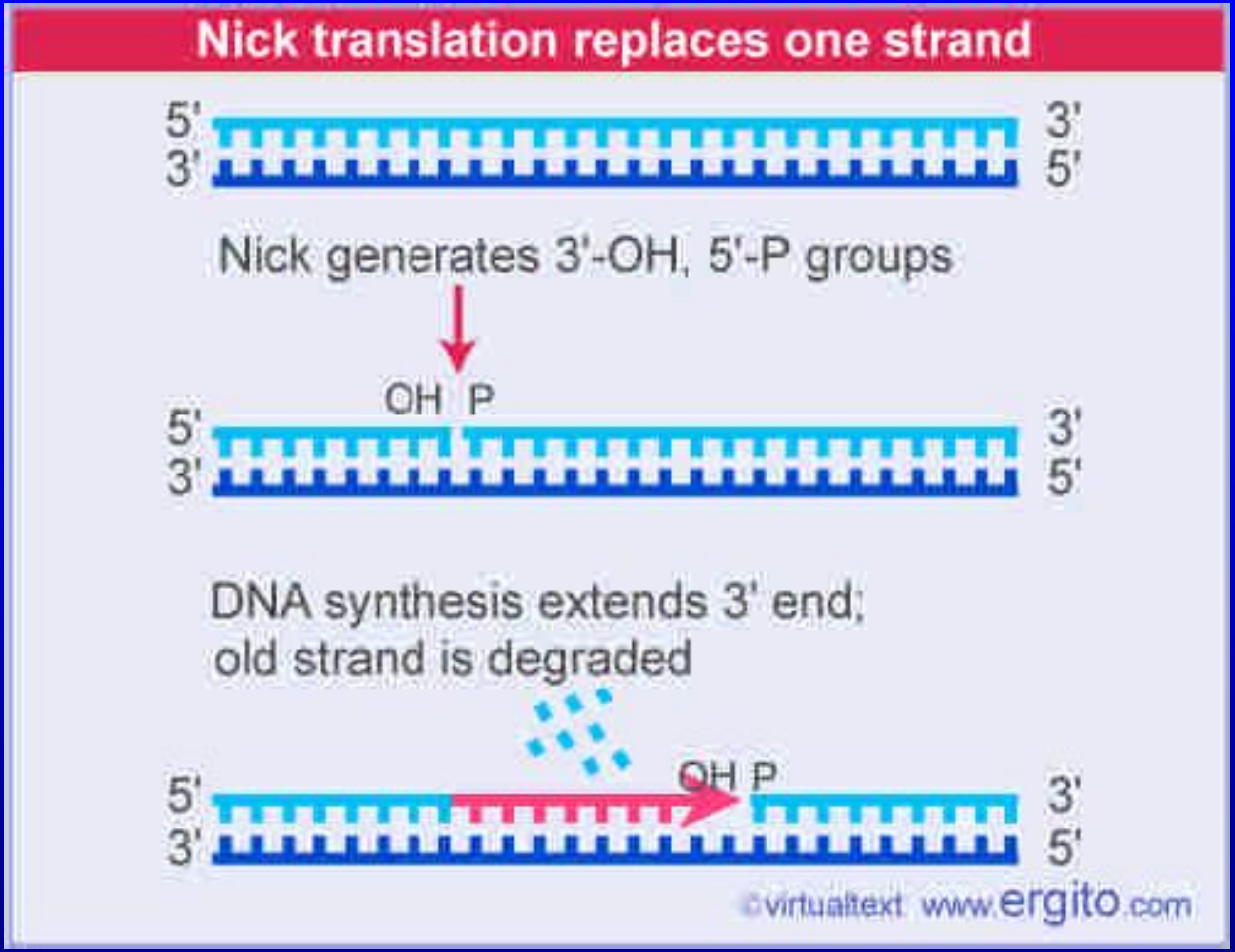


5′➔3′ Exonuclease Activity





Nick Translation



5'-3'exonuclease activities (35kD)

The functions of DNA pol I

- **Not major replicase**
- **Remove RNA primer**
- **DNA repair：remove TT dimer in UV damage**
- **Replace strand：take part in gene recombinant**
- **Nick translation**
- **Label probe**

DNA Polymerase II

5'–3' polymerase activity

3'–5' exonuclease activity

DNA polymerase II is required to restart a replication fork when its progress is blocked by damage in DNA.

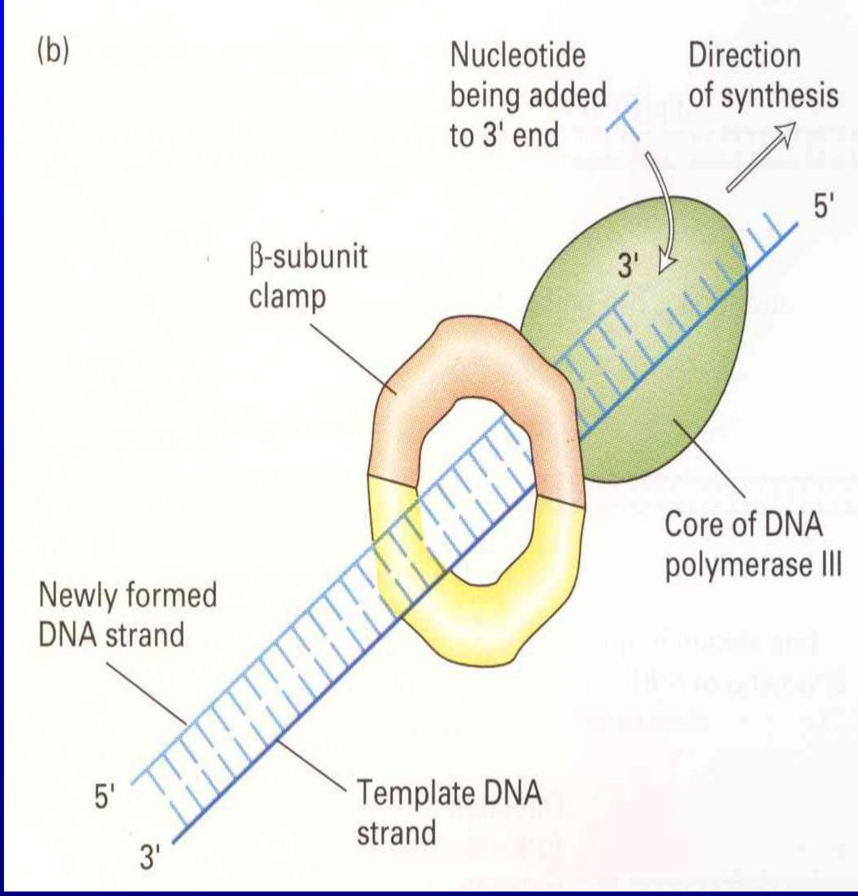
DNA polymerase III

Major replicase, high catalytic efficiency

subunits: α ε θ β γδδ'χφτ

（1）core of DNA pol III：

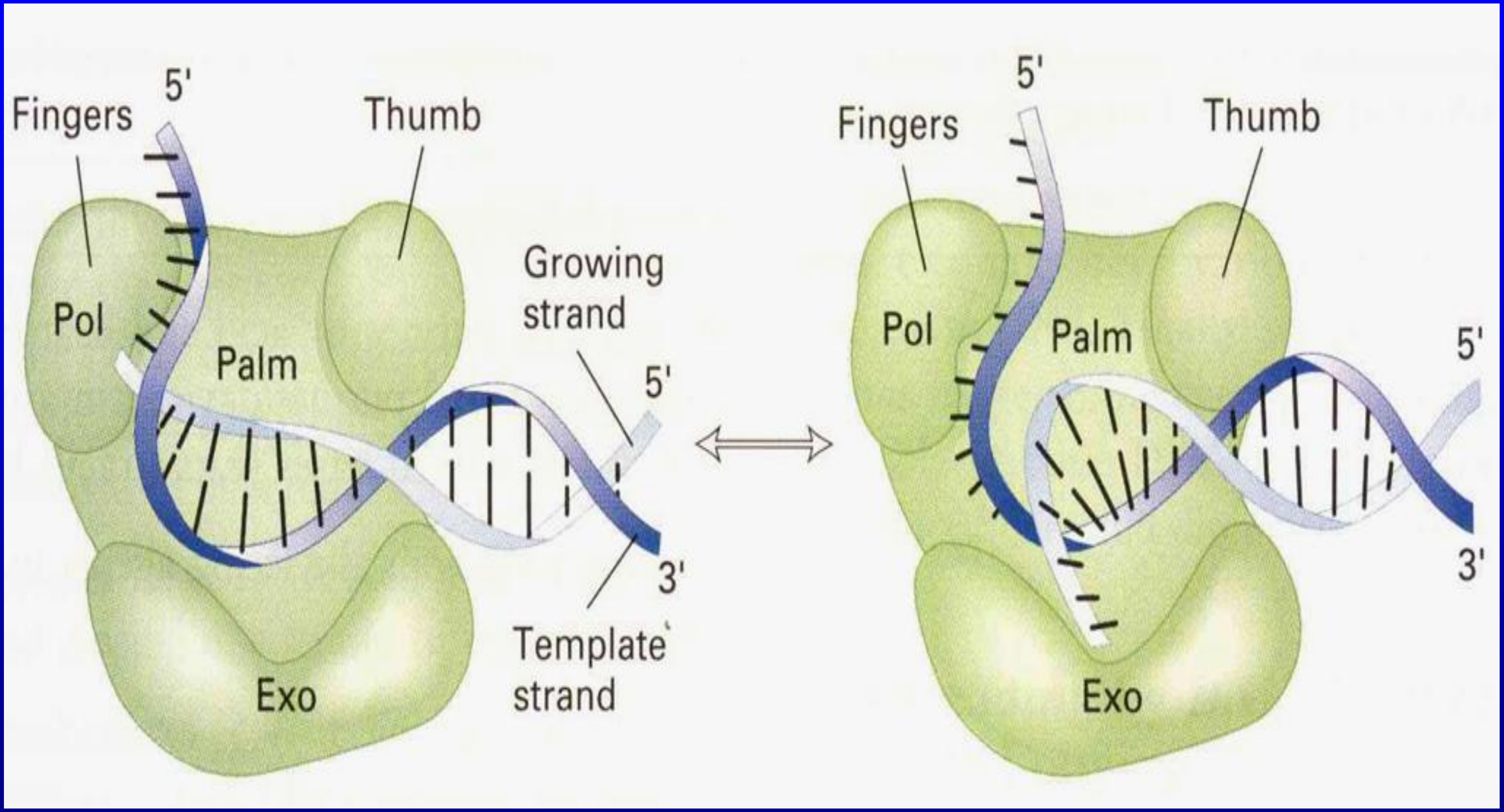
- α： DNA polymerase activity
- ε： 3'-5'exonuclease，fidelity control



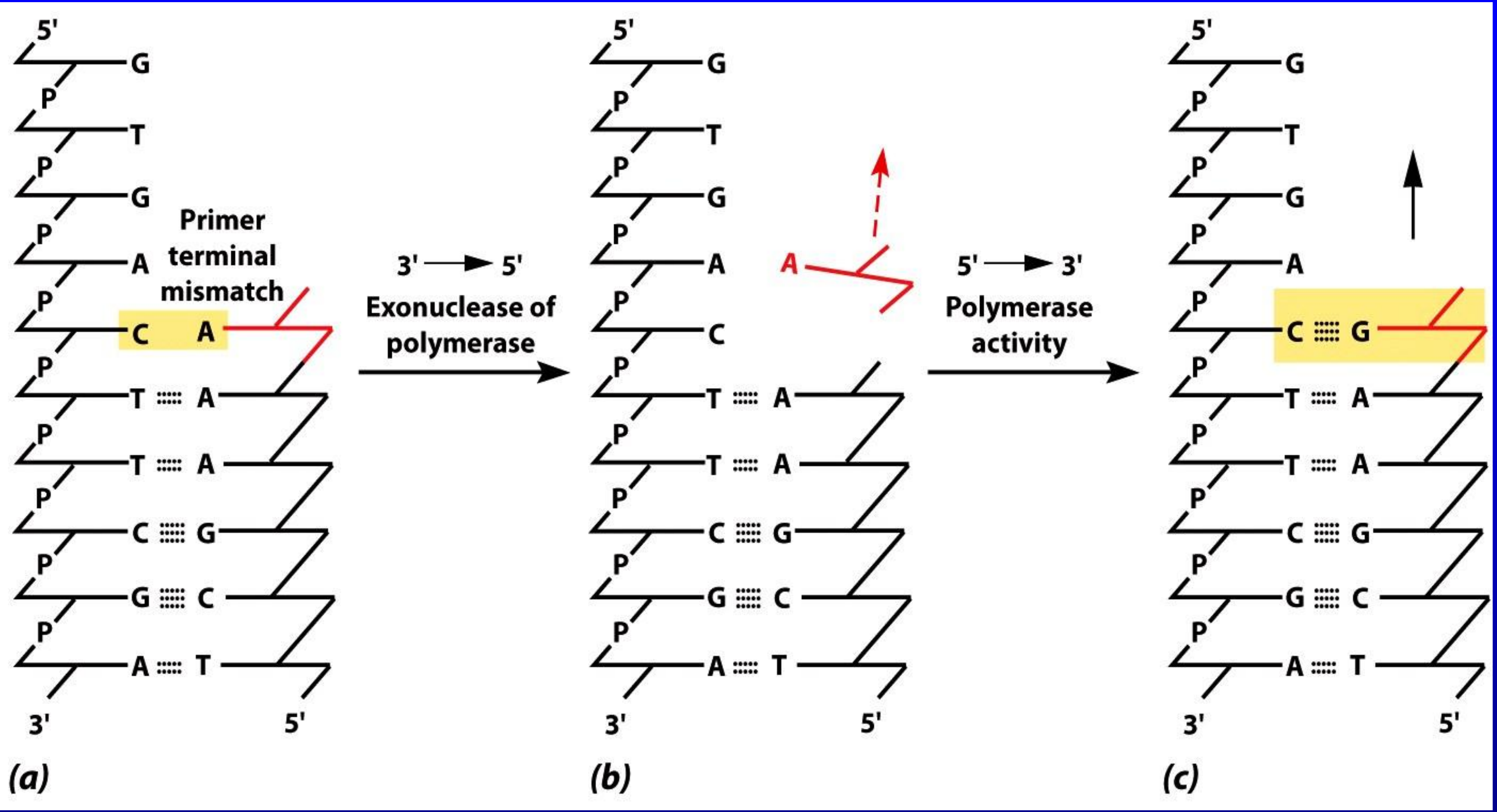
（2）β subunit clamp： help holoenzyme binding on DNA （20bp/s---- 750bp/s）

（3）γ complex： γ2δδ'χφ help β dimer binding on DNA

Schematic model of DNA Polymerase III



Proofreading



Proofreading by the 3’ → 5’ exonuclease activity of DNA polymerases during DNA replication.

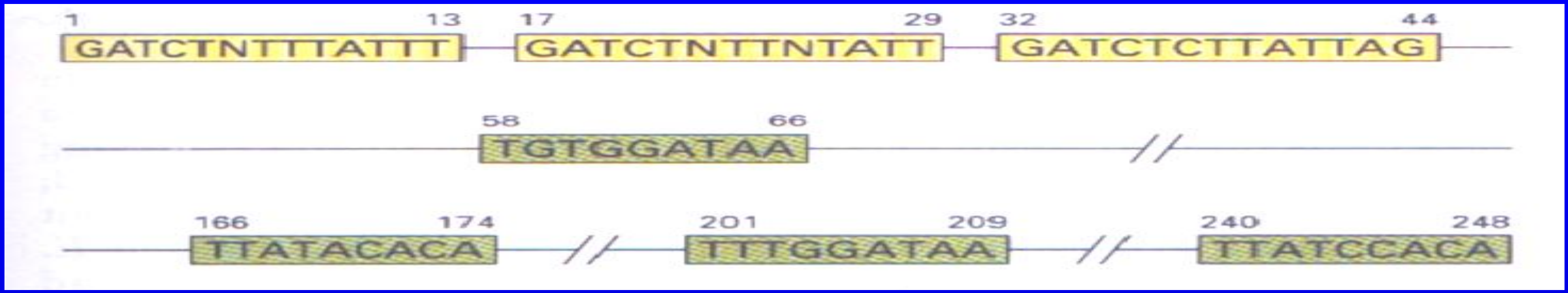
Properties of Three Bacterial DNA Polymerases

	I	II	III
Initiation of chain synthesis	-	-	-
5'-3' polymerization	+	+	+
3'-5' exonuclease activity	+	+	+
5'-3' exonuclease activity	+	-	-
Molecules of polymerase/cell	400	?	15
In vitro chain elongation rate	600	?	30000

Section Two

DNA replication in *E. coli*

Initiation of DNA replication



DnaA: binds to 9 bp repeat sequences at *oriC*, then acts at three A-T-rich 13 bp tandem repeats, and melts the DNA strands to form an open complex in the presence of ATP.

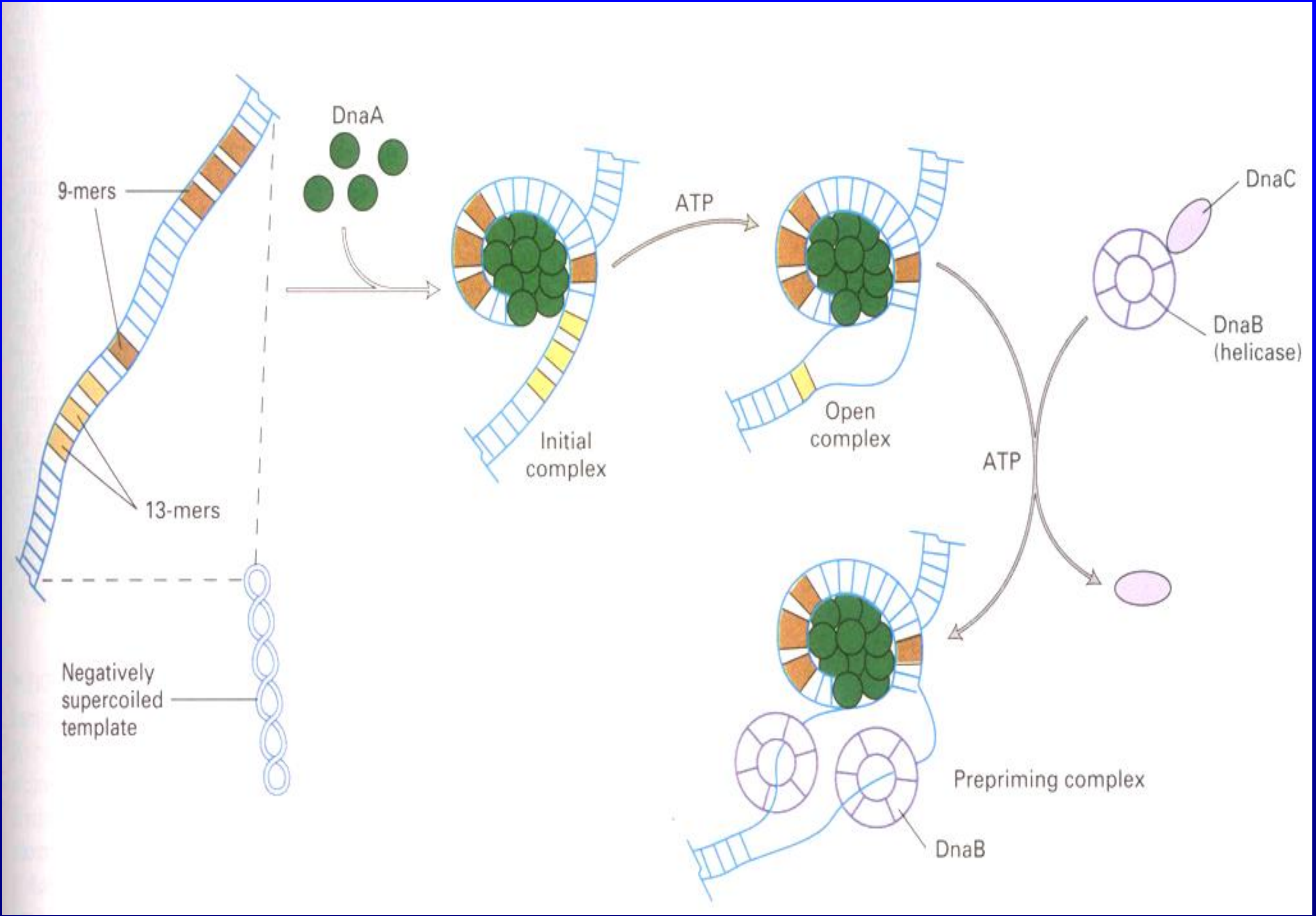
DnaB: extends the unwinding region with its helicase activity, and activates DnaG primase.

DnaC: binds to DnaB to form DnaB·DnaC complex, and then transfers DnaB to *OriC*. DnaC hydrolyzes ATP inorder to release DnaB.

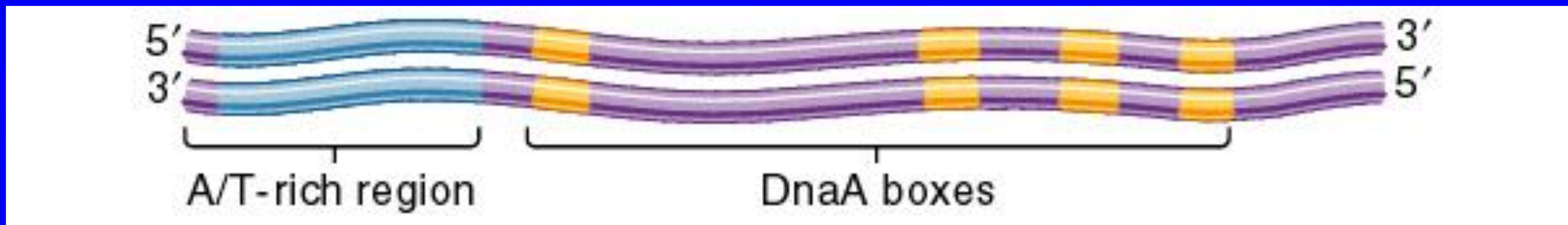
HU: a general DNA-binding protein in *E. coli*. Its presence is not absolutely required to initiate replication *in vitro*, but it stimulates the reaction.

Gyrase provides a swivel that allows one strand to rotate around the other; without this reaction, unwinding would generate torsional strain in the DNA.

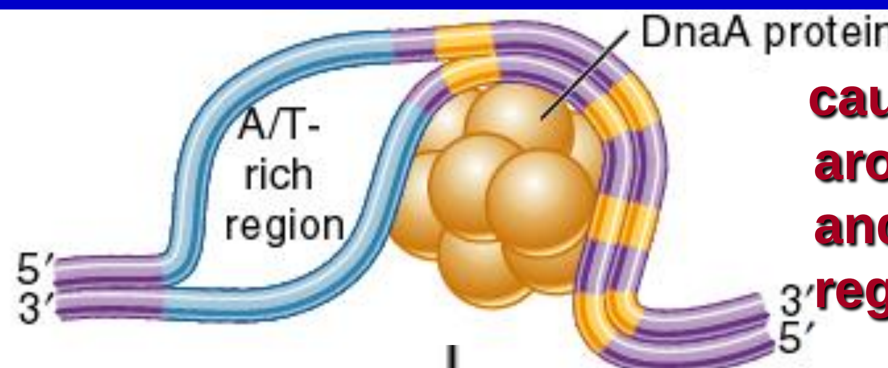
SSB stabilizes the single-stranded DNA as it is formed.


DnaA
9-mers
13-mers
Negatively
supercoiled
template
ATP
Initial
complex
Open
complex
DnaC
DnaB
(helicase)
ATP
Prepriming complex
DnaB

Initiation of Replication at oriC

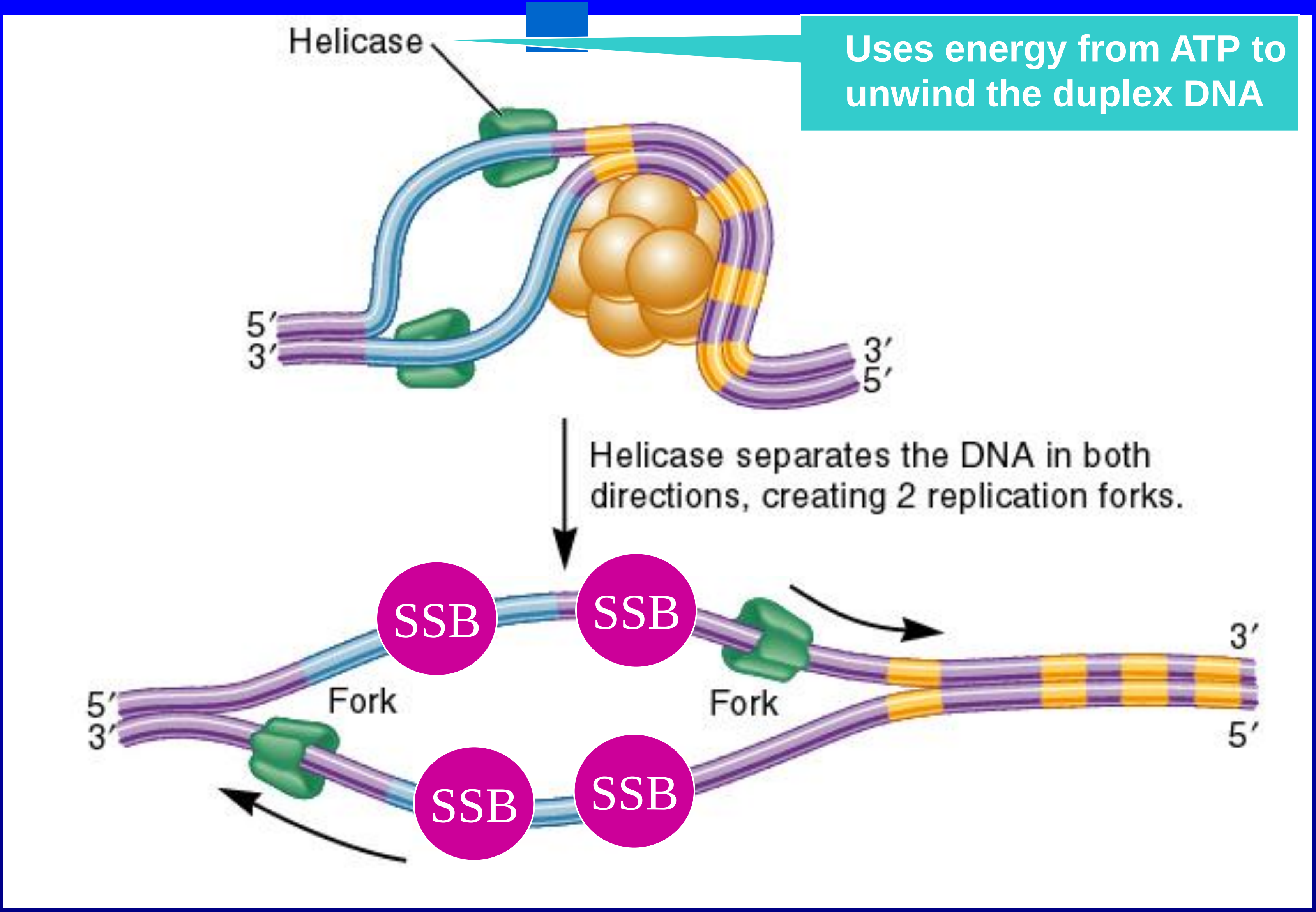


DNA replication is initiated by the binding of **DnaA proteins** to the **DnaA box sequences**



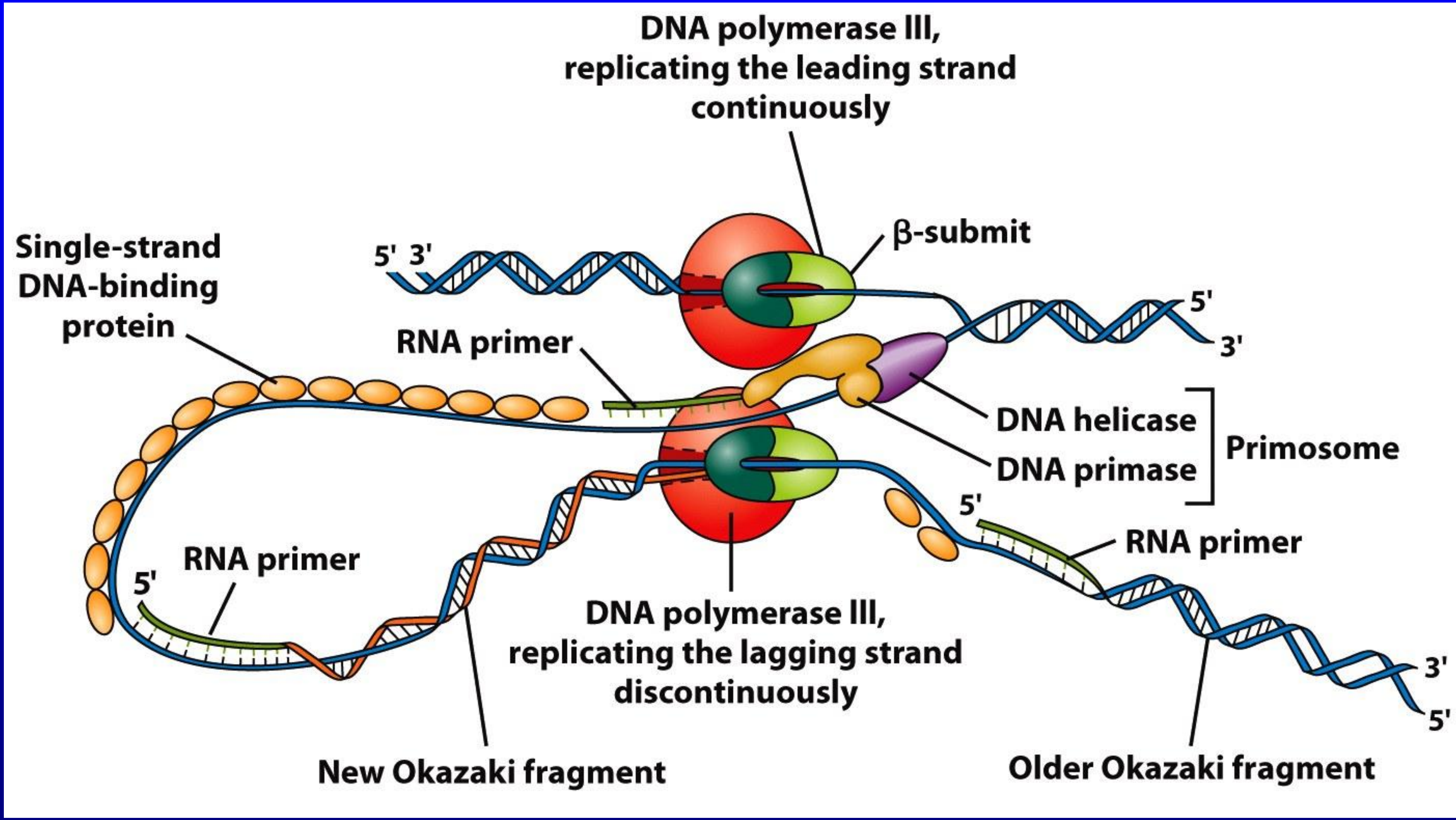
causes the region to wrap around the DnaA proteins and separates the AT-rich region

DnaB protein (helicase) binds to the origin

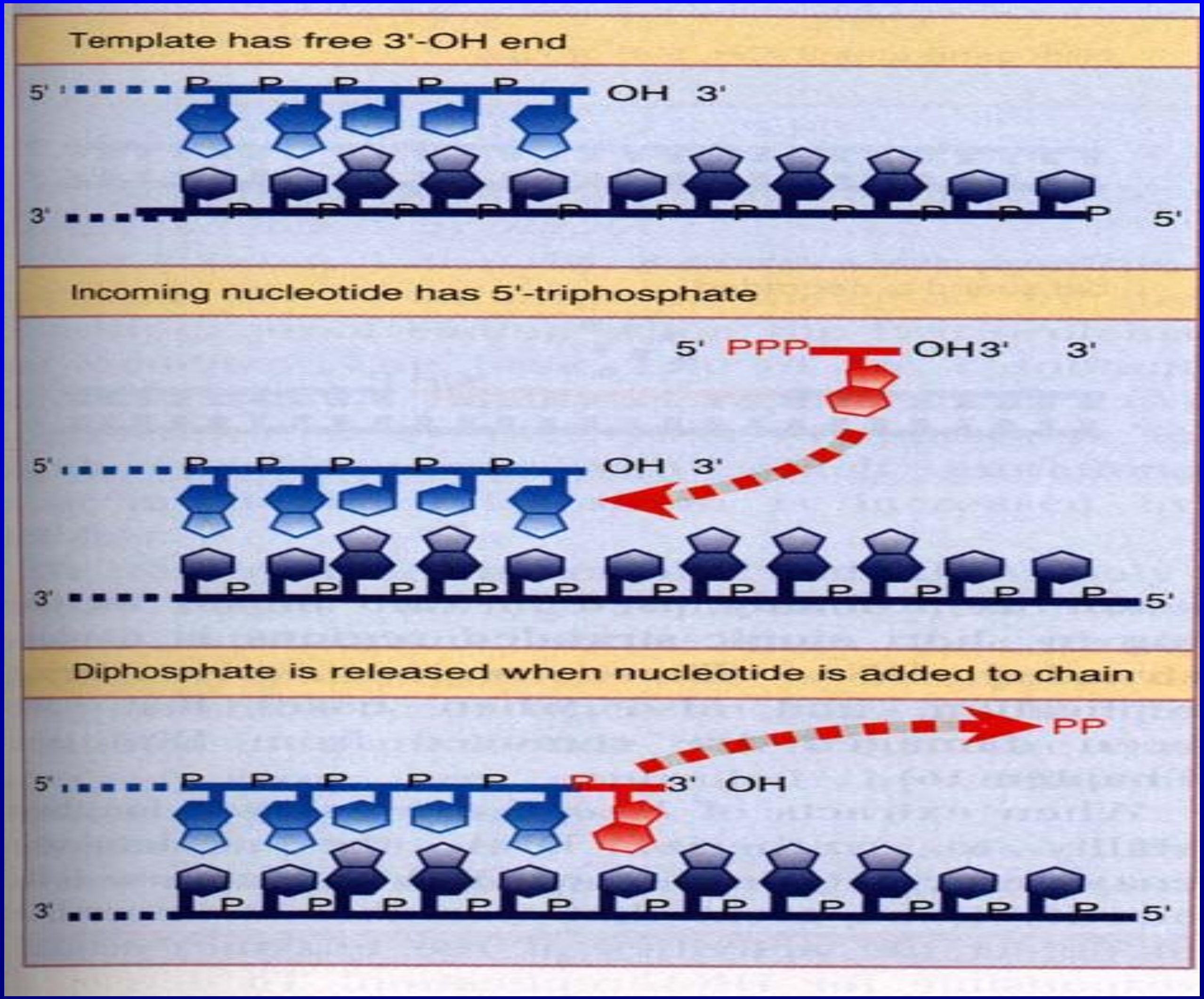


Uses energy from ATP to unwind the duplex DNA
Helicase
5′
3′
3′
5′
Helicase separates the DNA in both directions, creating 2 replication forks.
SSB
SSB
SSB
SSB
Fork
Fork
5′
3′
3′
5′

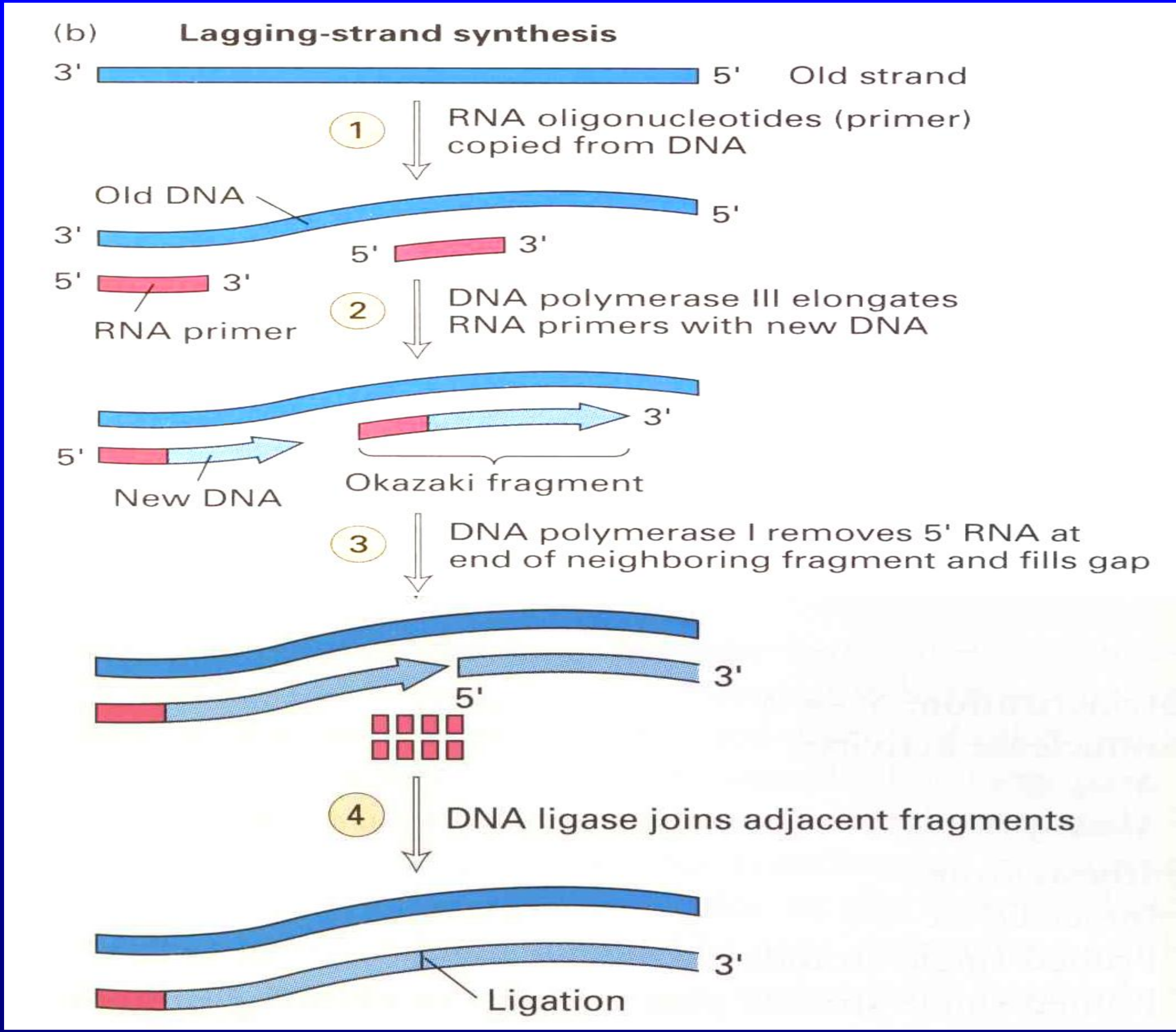
The *E. coli* Replisome（复制体）

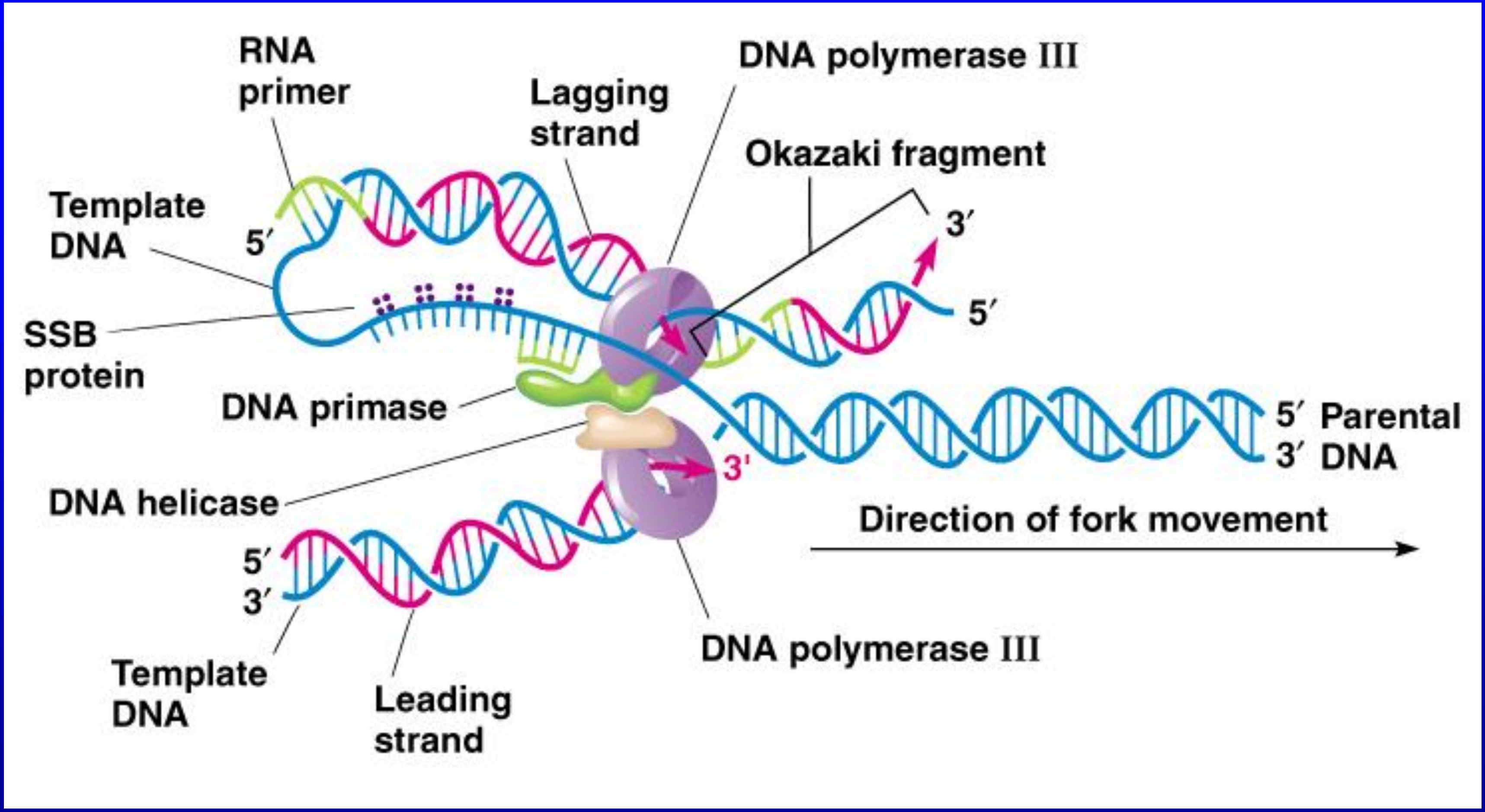


The elongation of leading strand



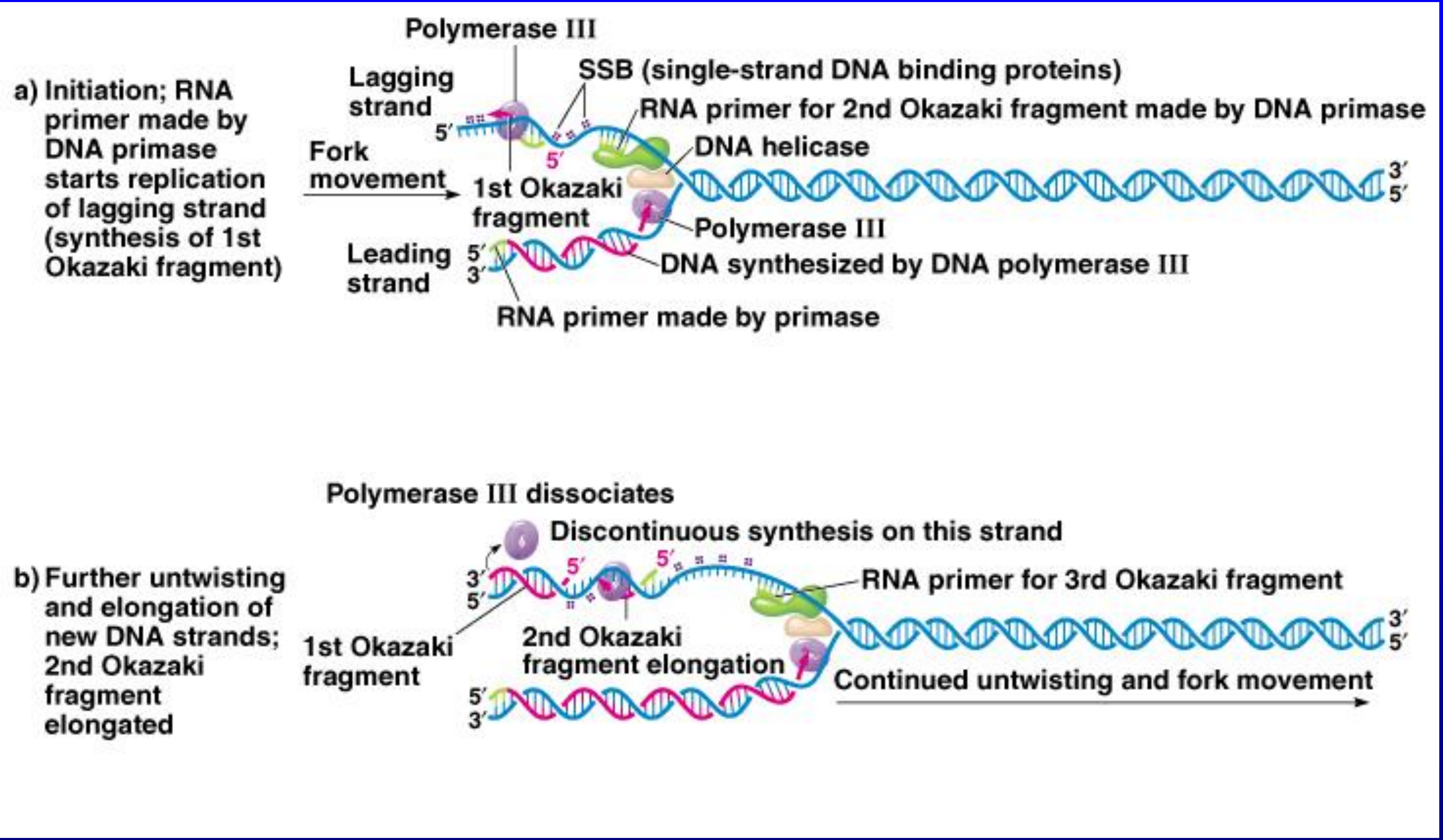
The elongation of lagging strand



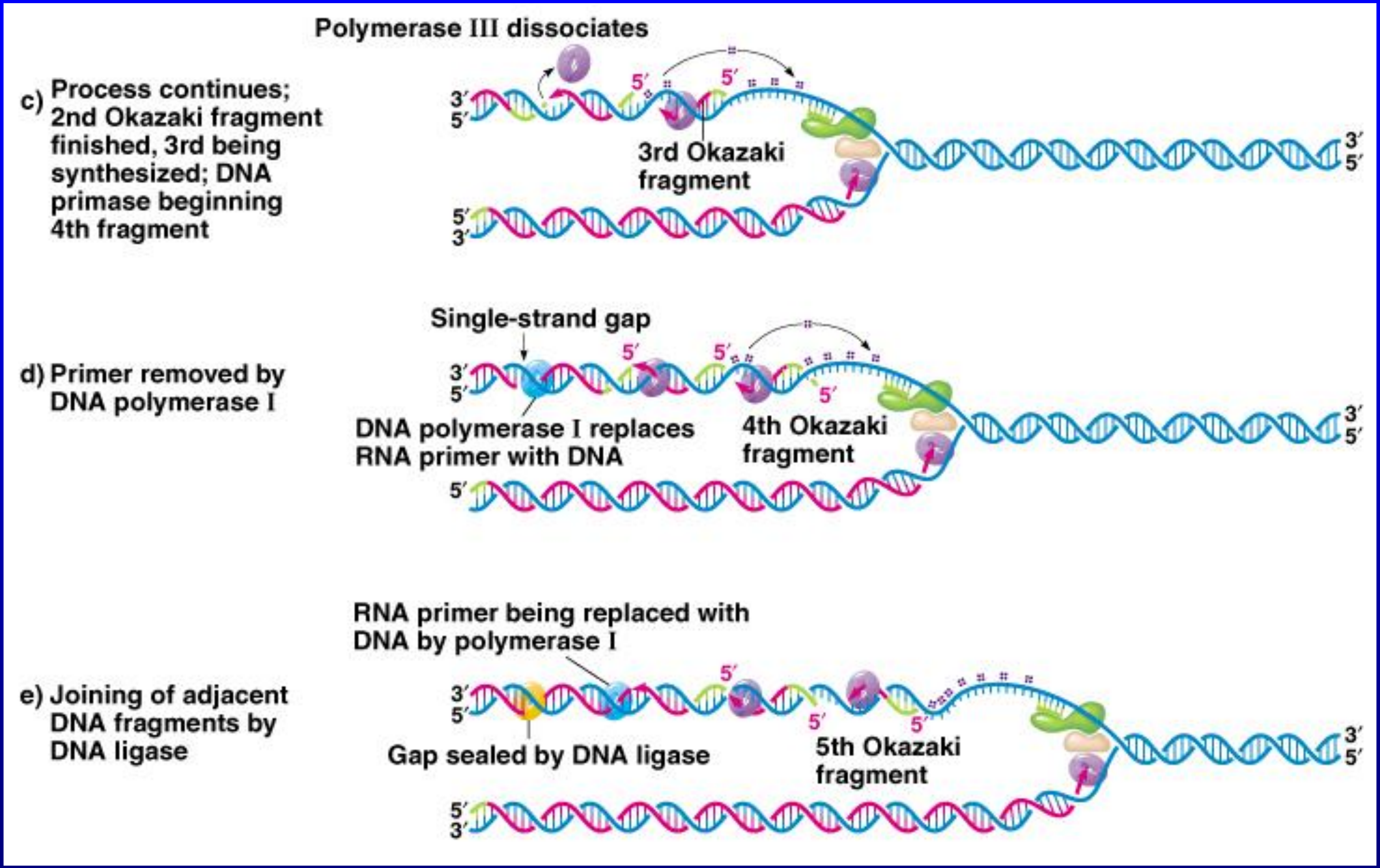


Looping the lagging strand to make both polymerases move in the same direction

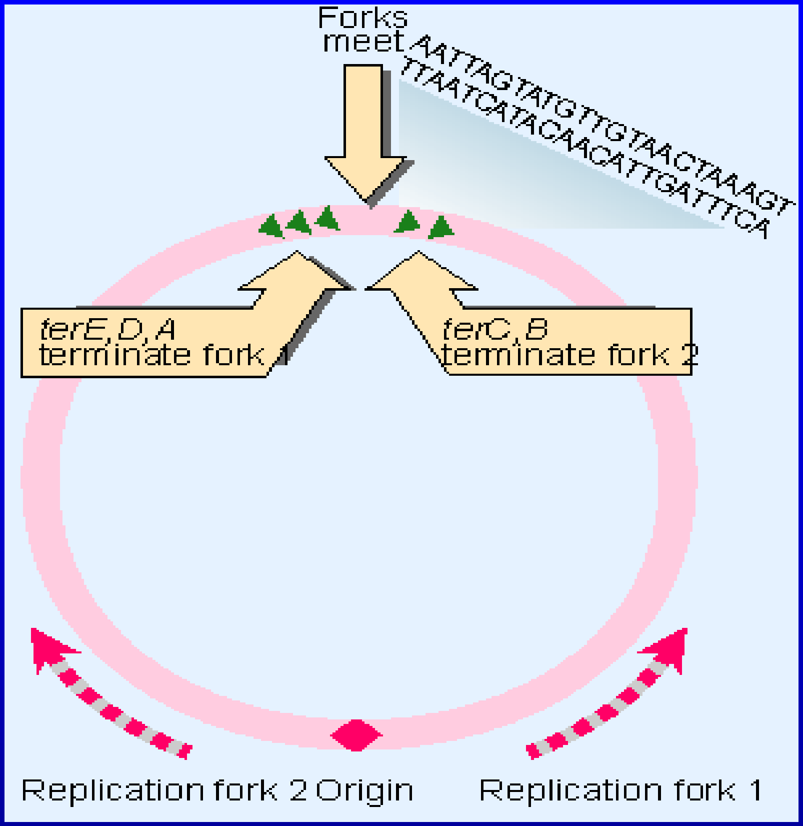
Model for the events occurring around a single replication fork of the *E. coli* chromosome



Model for the events occurring around a single replication fork of the *E. coli* chromosome (continued)



Termination in *E. coli*



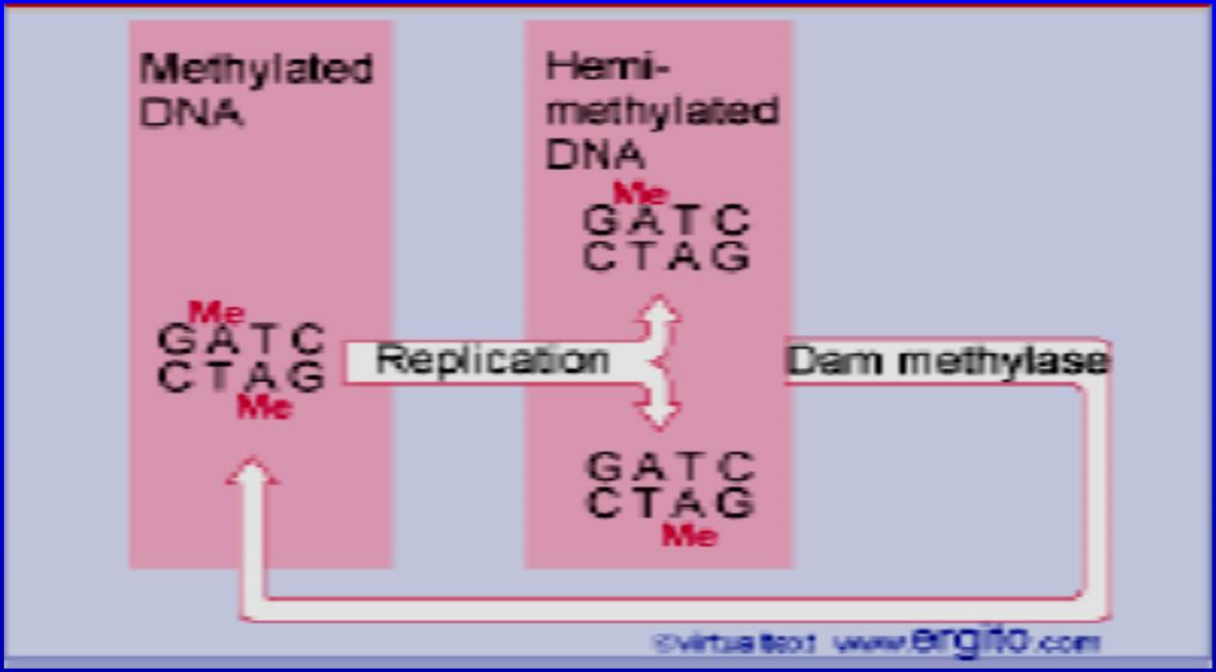
Sequences that cause termination are called ***ter*** **sites （~23 bp） which is recognized by Tus protein.**

Tus is a contra-helicase，inhibits helicase activity of DnaB and prevents the replication fork from proceeding。Tus also can dissociate replisome。

Summary of DNA replication

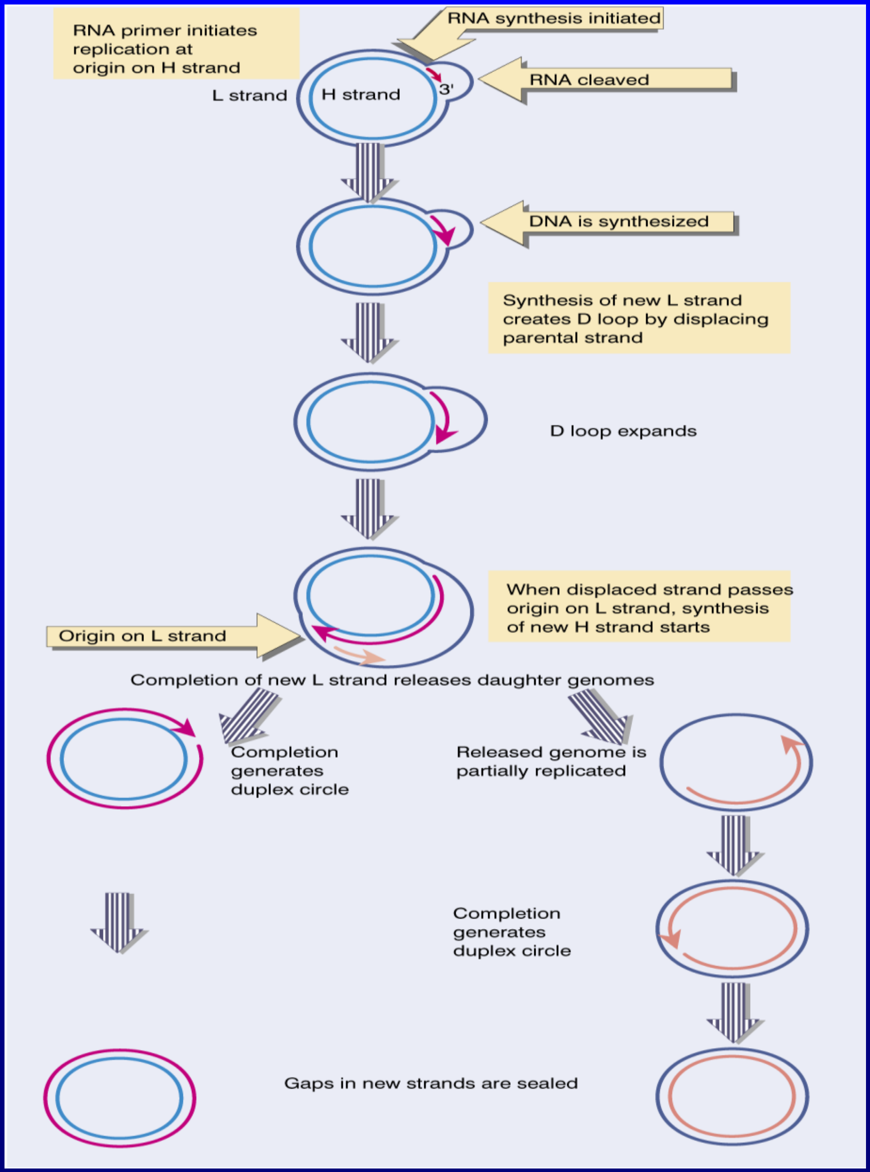
- **DNA replication is complex, requiring the participation of a large number of proteins.**
- **DNA synthesis is continuous on the progeny strand that is being extended in the overall 5'→3' direction, but is discontinuous on the other strand.**
- **New DNA chains are initiated by short RNA primers synthesized by DNA primase.**
- **The enzymes and DNA-binding proteins involved in replication assembled into a replisome at each replication fork and act in concert as the fork moves along the parental DNA molecule.**

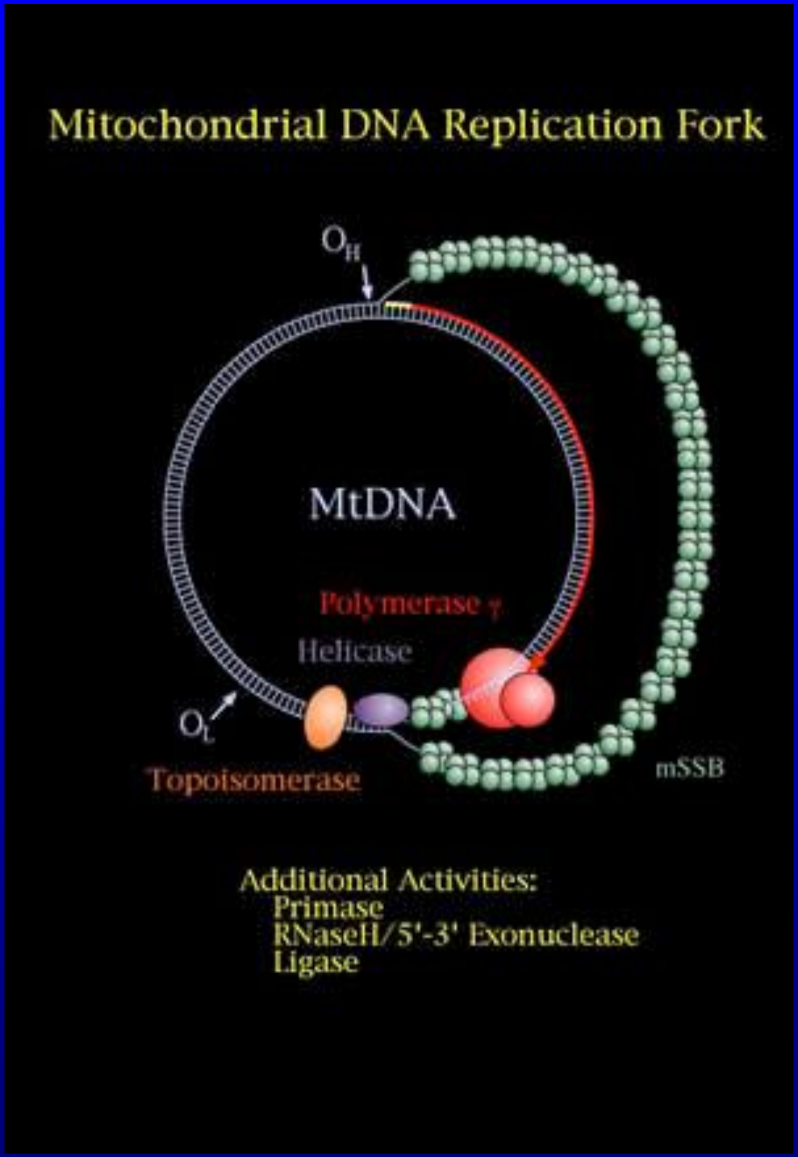
Methylation of the bacterial origin regulates initiation



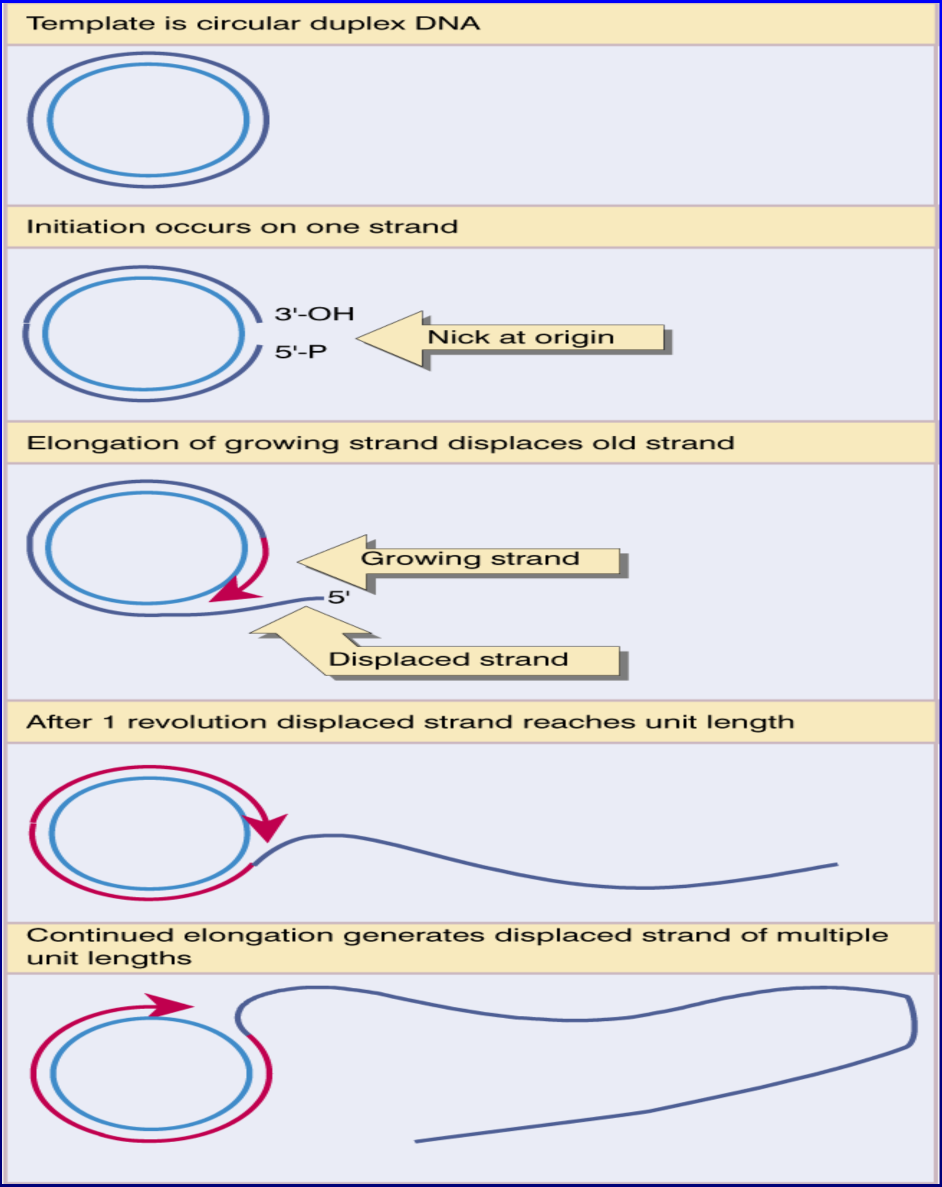
- *oriC* contains 11 repeats GATC that are methylated on adenine on both strands.
- Replication generates hemimethylated DNA.
- SeqA binds to hemimethylated GATC sites and inhibits Dam and DnaA binding to *oriC*

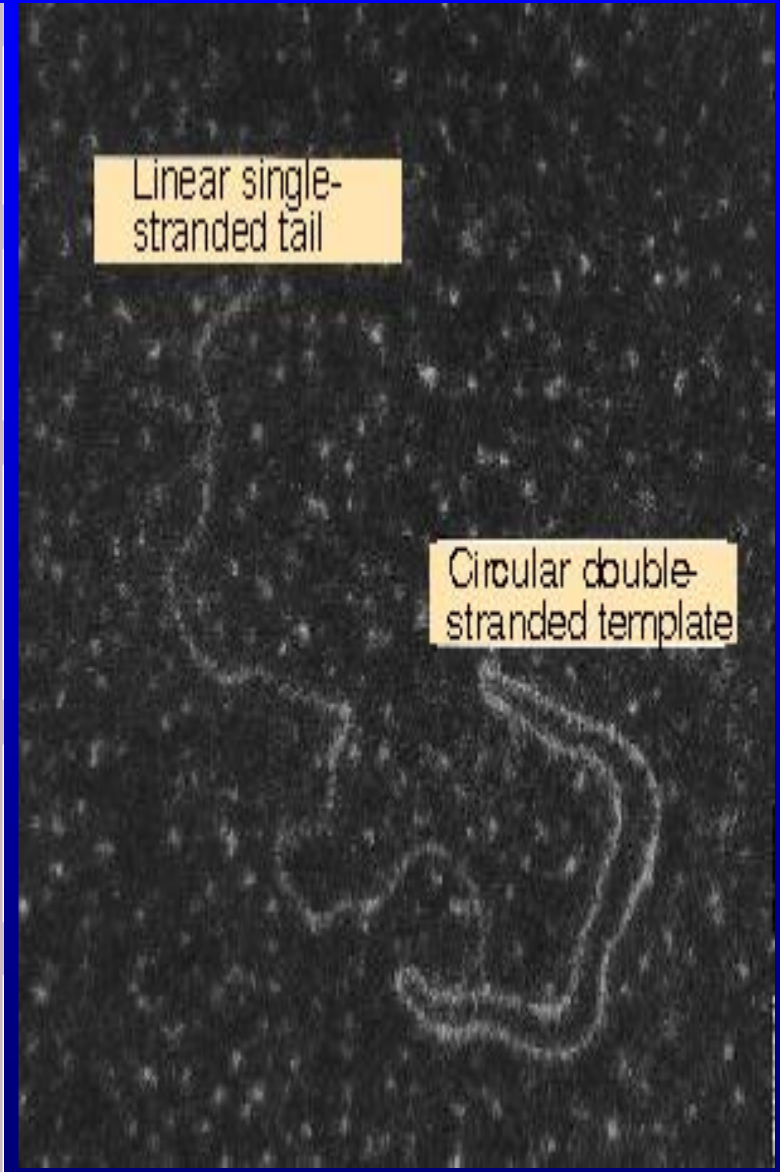
Mitochondrial DNA replication in D loop manner





Phage DNA replication in rolling circle manner





Circular DNA replication： θ