Part Ⅱ DNA biosynthesis and recombination

DNA biosynthesis

- **DNA replication**
- **Reverse transcription**

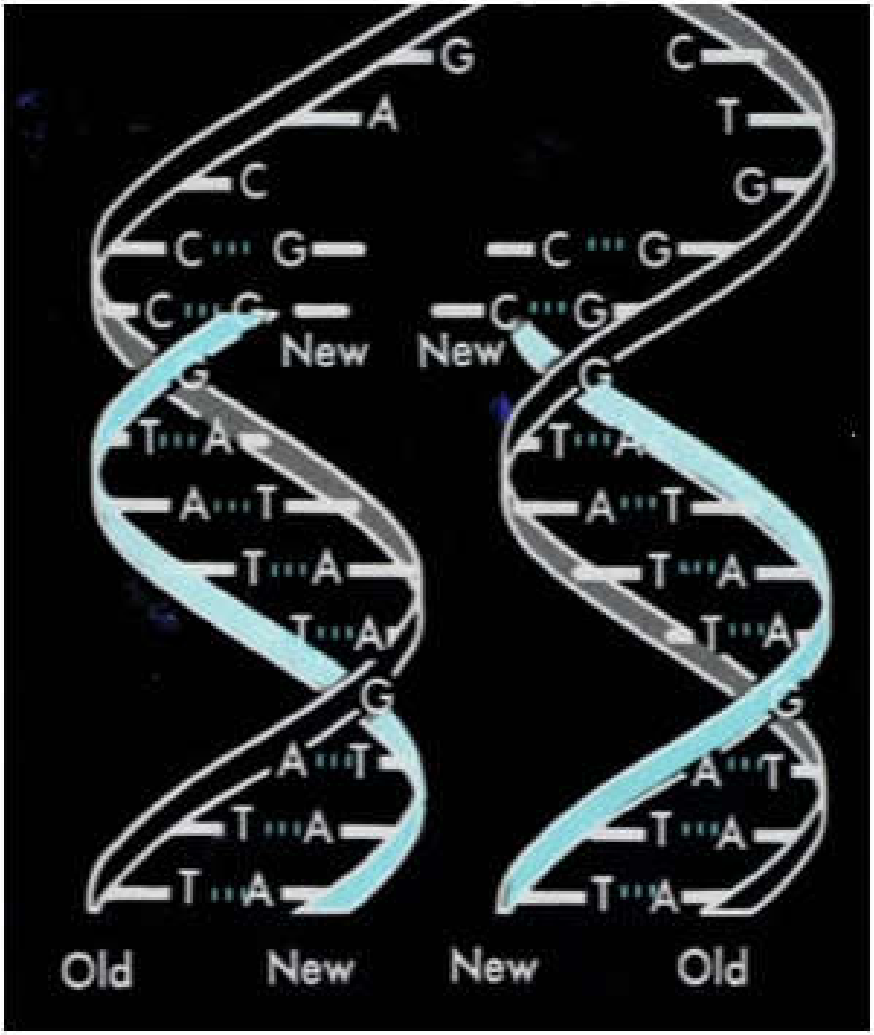
Chapter 2 DNA replication

1. DNA replication: an overview

DNA double helix

Base sequences are complementary.

1.1 Semi-conservative mechanism



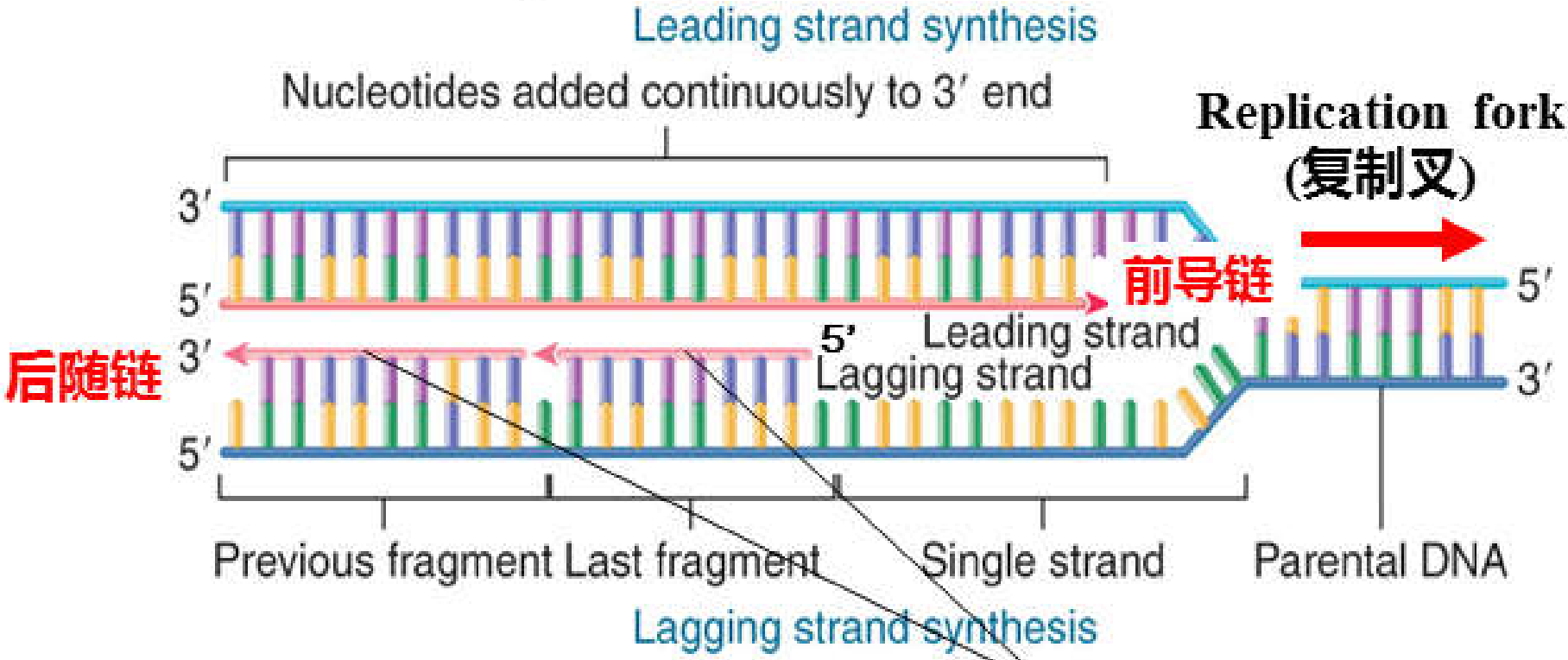
半保留复制

During replication, the strands of the DNA double helix separate and each acts as a template to direct the synthesis of a complementary daughter strand.

1.2 Semi-discontinuous replication

半不连续复制

- Two strands of DNA are antiparallel (反平行);
- DNA can only be synthesized in a 5'→3' direction.



1000-2000 nt long in prokaryotes;
and 100-200 nt long in eukaryotes.

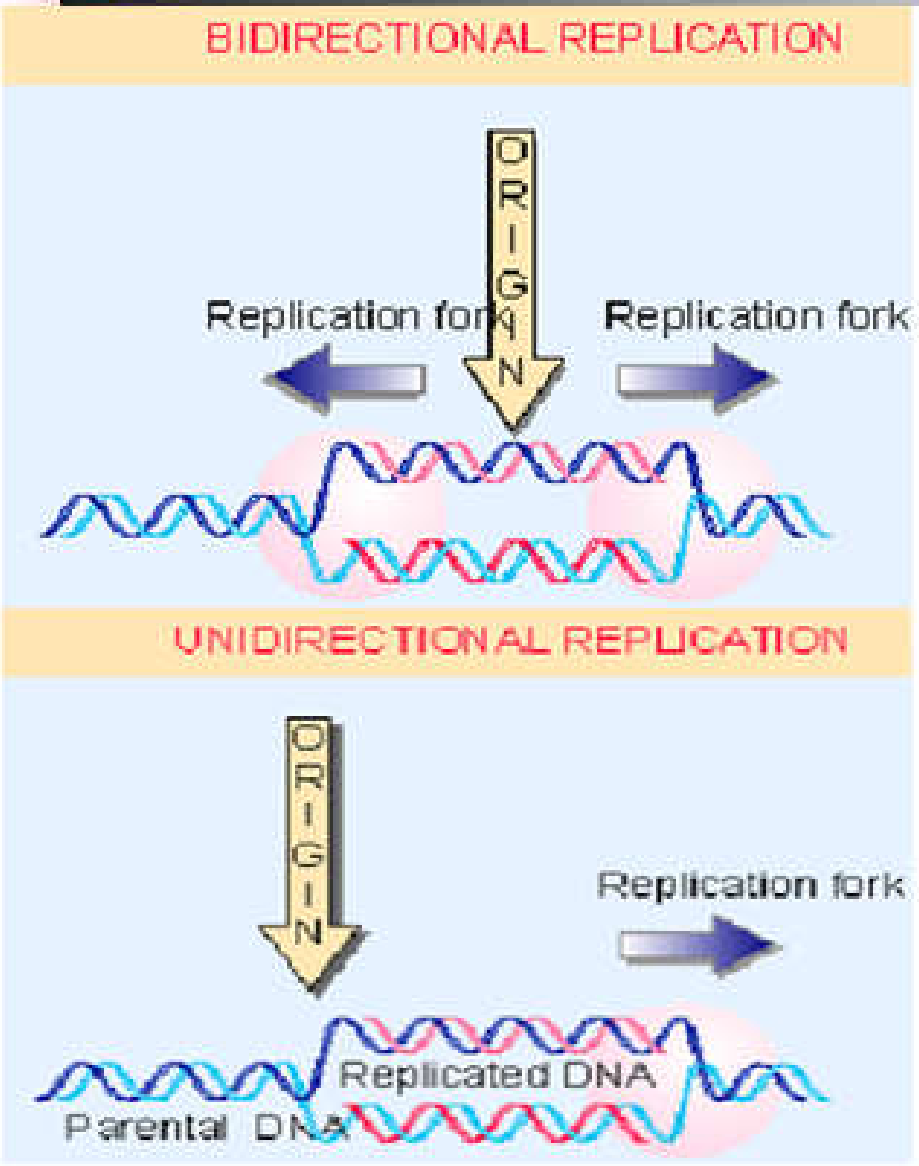
Okazaki fragments
(冈崎片段)

1.3 Origins, termini, and replicons

1.3.1 Origins（复制起点）

- **Origin** is the initiation point of DNA replication. (A **fixed region** contains **AT-rich** sequences).
- All prokaryotic chromosomes, bacteriophage (噬菌体) and viral (病毒的) DNA molecules have a **single origin**.
- **Eukaryotic** chromosomes have **multiple** origins.

复制方向

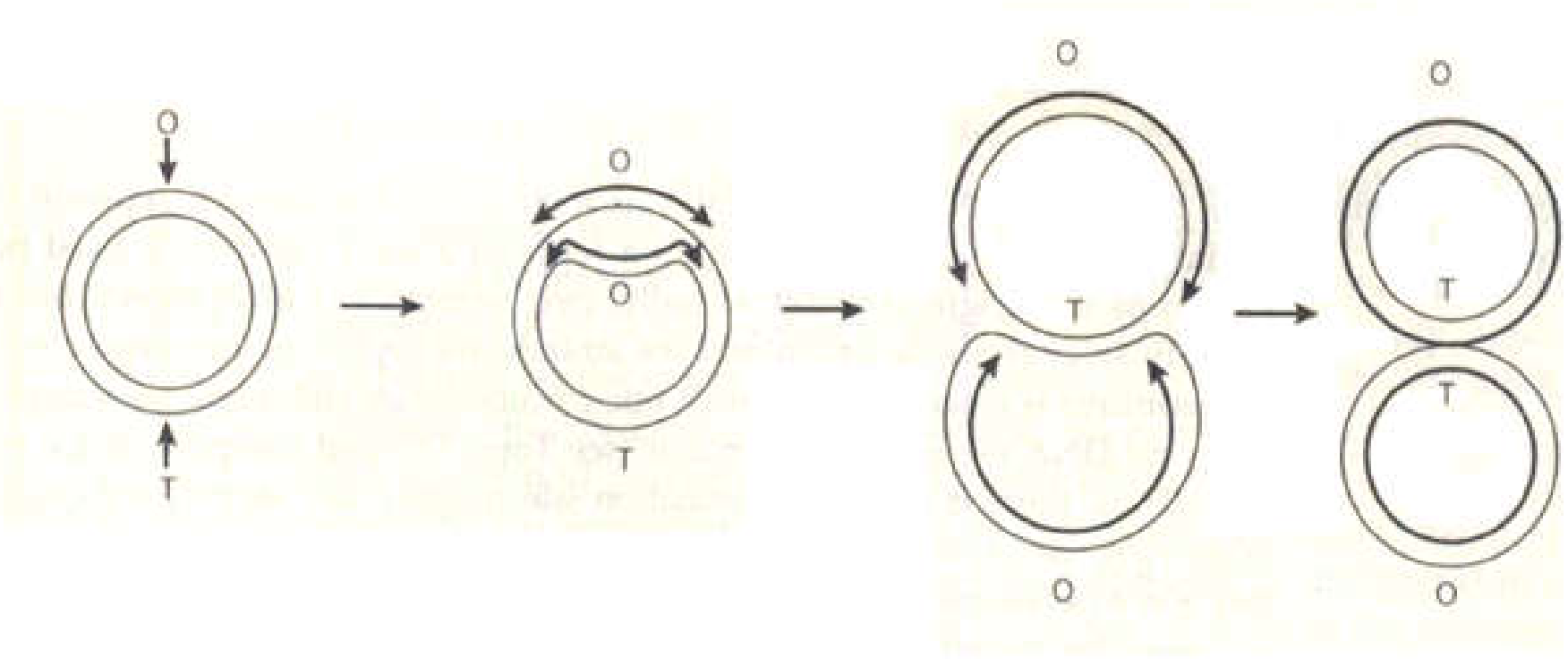


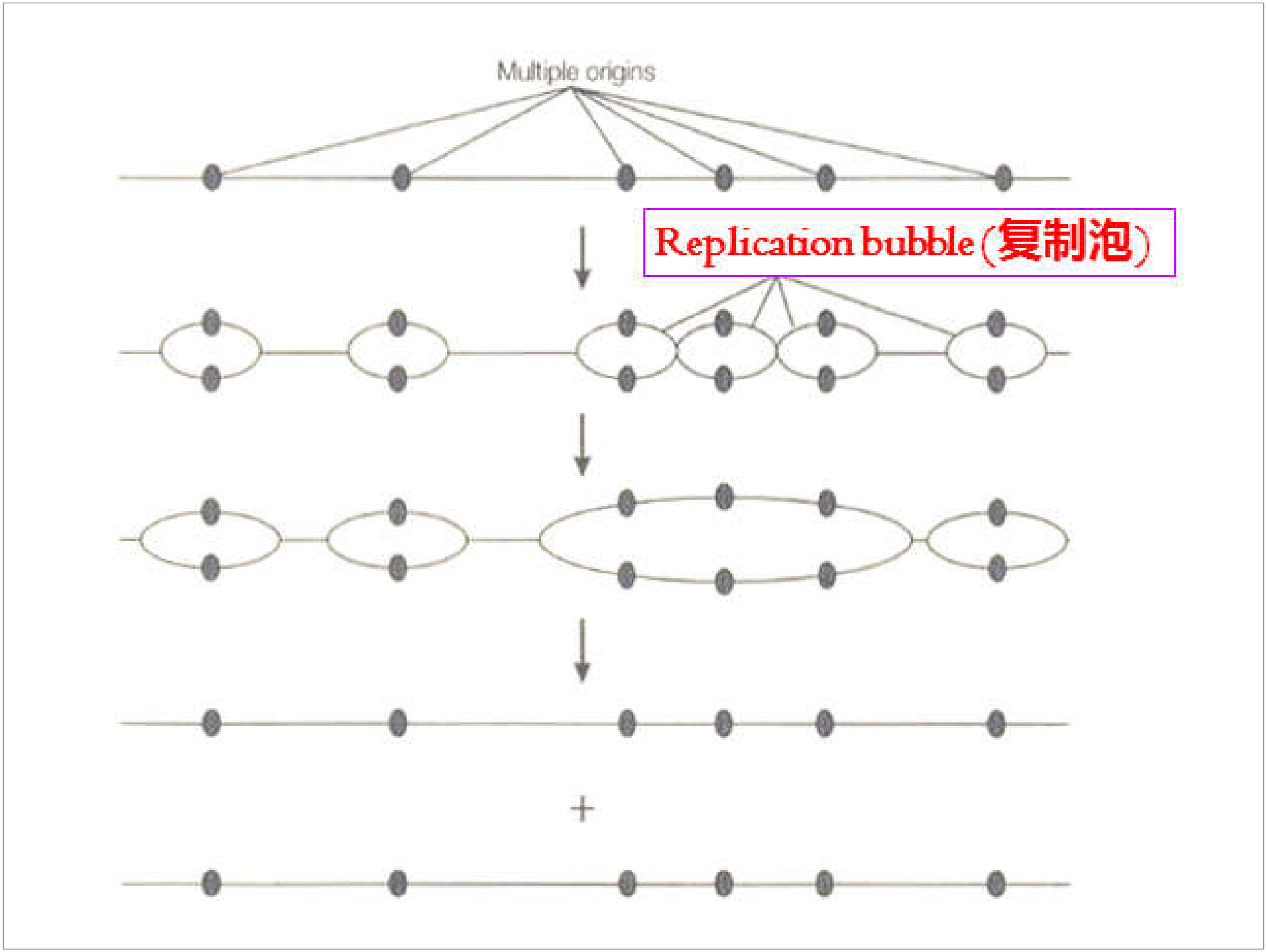
Usually, two replication forks proceed bidirectionally (双向，从起始点向两个方向进行) away from the origin.

Unidirectional (单向，从起始点向一个方向进行)

1.3.2 Termini（复制终点）

- **Terminus is the ending point of DNA replication.**




Multiple origins
Replication bubble (复制泡)

1.3.3 Replicons

- **Replicon** is any piece of DNA which replicates as a **single unit**.
  DNA上任何一个独立复制单位称为**复制子**。
- **Each replicon contains only one origin.**
- All **prokaryotic** chromosomes, **bacteriophage** and **viral DNA** molecules comprise a **single replicon**.
- **Eukaryotic** chromosomes have **multiple replicons.**

1.4 Key proteins involved in DNA replication

1.4.1 DNA Polymerase (聚合酶)

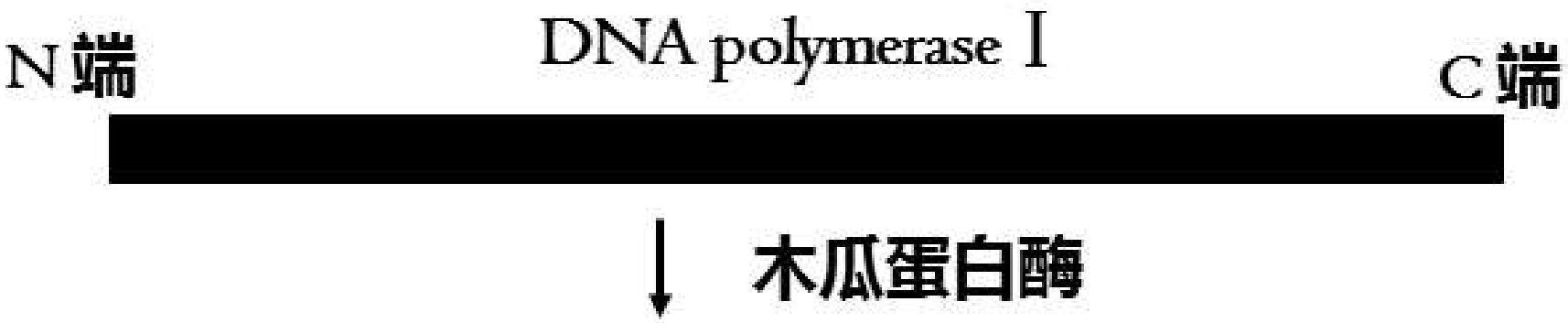
(1) Properties of DNA polymerase

DDDP

- Substrates：dNTPs (substrate/energy provider)
- Cofactor (辅因子): Mg^{2+} (for enzyme activity)；
- Template: single-stranded DNA (ssDNA)；
- Primer (引物) : RNA (with a free 3'-OH that the enzyme can be extend);
- Direction of DNA synthesis: 5'→3'

(2) DNA polymerases in prokaryotes (*E. coli*)

Properties and functions	*E. coli* DNA Polymerase		
	DNA Pol Ⅰ	DNA Pol Ⅱ	DNA Pol Ⅲ
Subunits	1	1	10
Molecules / cell	400	17-100	10-20
Rate (nt/s)	16~20	40	250~1000
5'→3' polymerase activity	Yes	Yes	Yes
5'→3' exonuclease activity (外切酶活性)	Removal of RNA primers	No	No
3'→5' exonuclease activity	Proofreading (校正)	Proofreading	Proofreading
Main functions	DNA synthesis Repair	Repair	Replicase



小片段

323个氨基酸

5′→3′核酸外切酶活性

大片段/Klenow片段

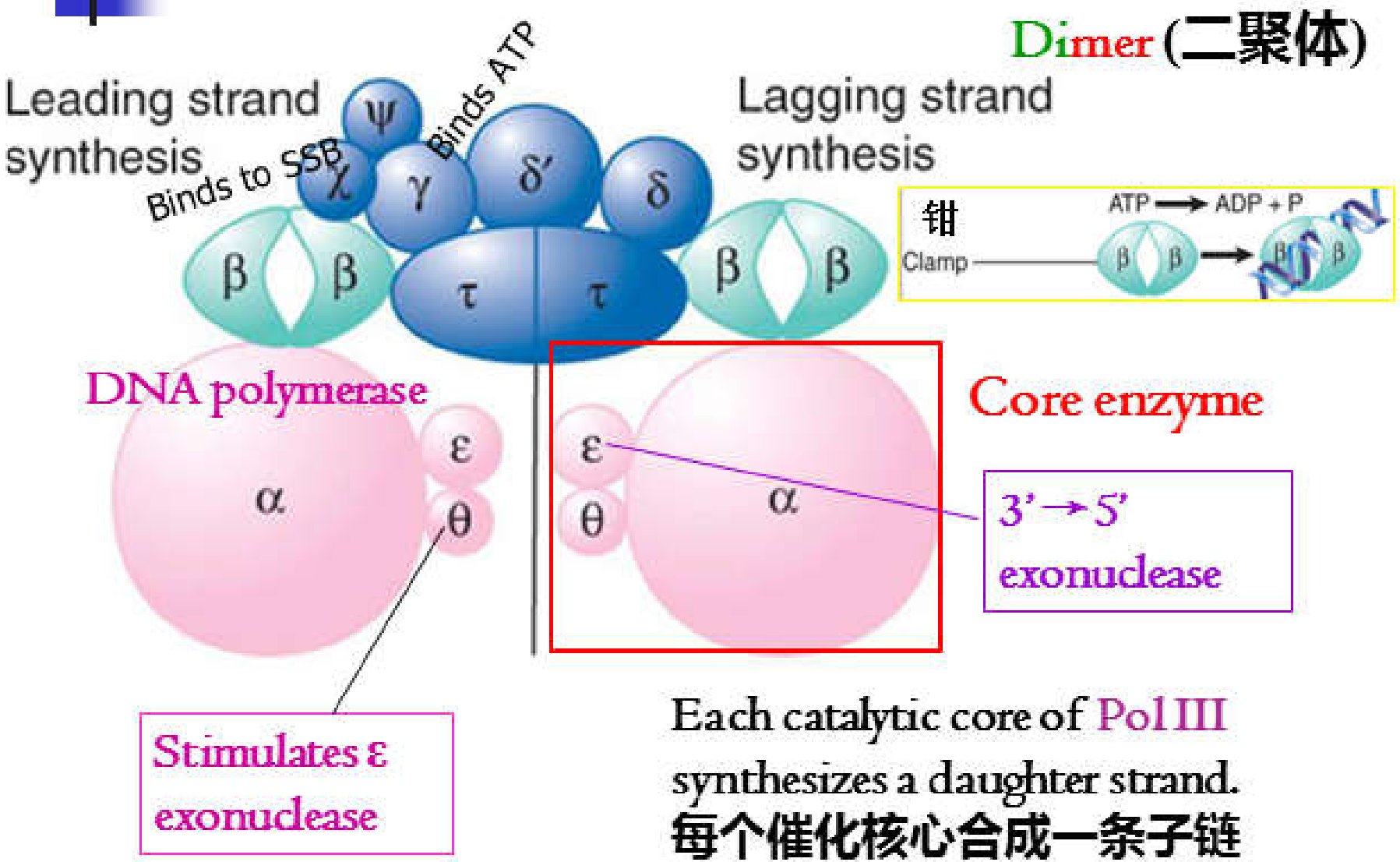
604个氨基酸

DNA聚合酶活性

3′→5′核酸外切酶活性

- Klenow片段是实验室合成DNA探针，进行分子生物学研究中常用的工具酶。

DNA polymerase Ⅲ holoenzyme (全酶)



(3) DNA polymerases in eukaryotes

Properties	Pol α	Pol β	Pol γ	Pol δ *	Pol ε *
Subunits	4	I	2	4	4
Intracellular location	Nucleus	Nucleus	Mitochondria	Nucleus	Nucleus
5'→3' polymerase	+	+	+	+	+
5'→3' exonuclease	-	-	-	-	-
3'→5' exonuclease	-	-	+	+	+
Functions	Priming 引发	DNA repair	Replication	Elongation of lagging strand	Elongation of leading strand

Yeast DNA Polymerase ε Participates in Leading-Strand DNA Replication

Zachary F. Pursell,[1] Isabelle Isoz,[2] Else-Britt Lundström,[2] Erik Johansson,[2] Thomas A. Kunkel[1]*

Multiple DNA polymerases participate in replicating the leading and lagging strands of the eukaryotic nuclear genome. Although 50 years have passed since the first DNA polymerase was discovered, the identity of the major polymerase used for leading-strand replication is uncertain. We constructed a derivative of yeast DNA polymerase ε that retains high replication activity but has strongly reduced replication fidelity, particularly for thymine-deoxythymidine 5′-monophosphate (T-dTMP) but not adenine-deoxyadenosine 5′-monophosphate (A-dAMP) mismatches. Yeast strains with this DNA polymerase ε allele have elevated rates of T to A substitution mutations. The position and rate of these substitutions depend on the orientation of the mutational reporter and its location relative to origins of DNA replication and reveal a pattern indicating that DNA polymerase ε participates in leading-strand DNA replication.

Replication of the eukaryotic nuclear genome requires DNA polymerase α to initiate synthesis at origins and to initiate synthesis of Okazaki fragments on the lagging strand, allowing DNA polymerases δ (pol δ) and ε (pol ε) to then perform the bulk of chain elongation (*1*, *2*). Pol δ is implicated in lagging-strand replication (*1*), but the identity of the polymerase(s) that replicates the leading strand is unknown (*1*, *2*). Null alleles of the *POL2* (pol ε) and *POL3* (pol δ) genes are uninformative for identifying the leading-strand polymerase, because both genes are essential for normal replication. To retain replication activity while generating a distinct mutational signature in vivo that allows assignment of pol ε to leading- and/or lagging-strand replication in yeast cells, we substituted glycine for Met^{644} at the *Saccharomyces cerevisiae* pol ε active site. Yeast pol ε with the $Met^{644}Gly$ change retains 44% of wild-type polymerase activity (Fig. 1A) and retains full 3′ exonuclease activity (Fig. 1B). A haploid *pol2-M644G* yeast strain grows at a rate similar to a *POL2* strain (Fig. 1C), indicating that $M^{644}G$ pol ε retains substantial replicative capacity. In both its exonuclease-proficient (Fig. 1D) and exonuclease-deficient forms (Fig. 1E), $M^{644}G$ pol ε synthesizes DNA in vitro with reduced fidelity in comparison with wild-type (i.e., Met^{644}) pol ε (Fig. 1 and table S1) (*3*), i.e., it is defective in discriminating against deoxynucleotide triphosphate (dNTP) misinsertion. Even the exonuclease-

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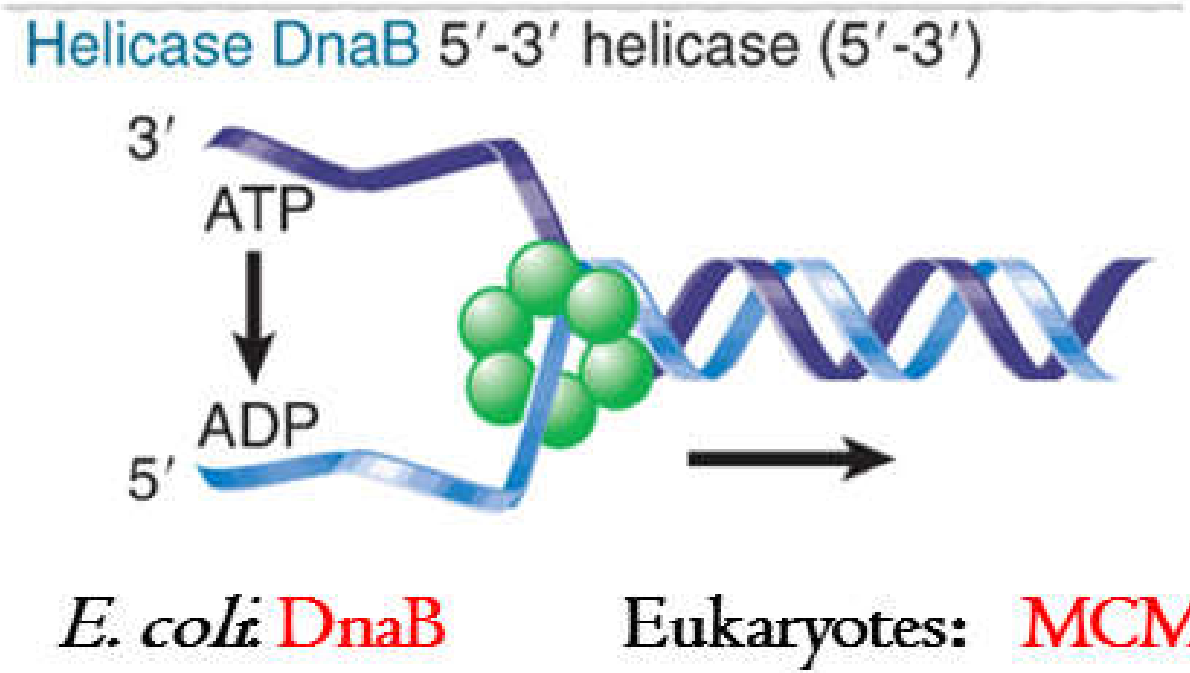
Weaver et al. Molecular Biology (Fifth Edition), 2012. P650

Lewin's GENES XI, 2014. P321-322

Watson et al. Molecular Biology of the Gene (Seventh Edition), 2014. P278

1.4.2 DNA helicase (解旋/链酶)

DNA helicase - the enzyme that uses the chemical energy of ATP to unwind (解旋/链) the two parental DNA strands at the replicating fork.



E. coli: DnaB Eukaryotes: MCM

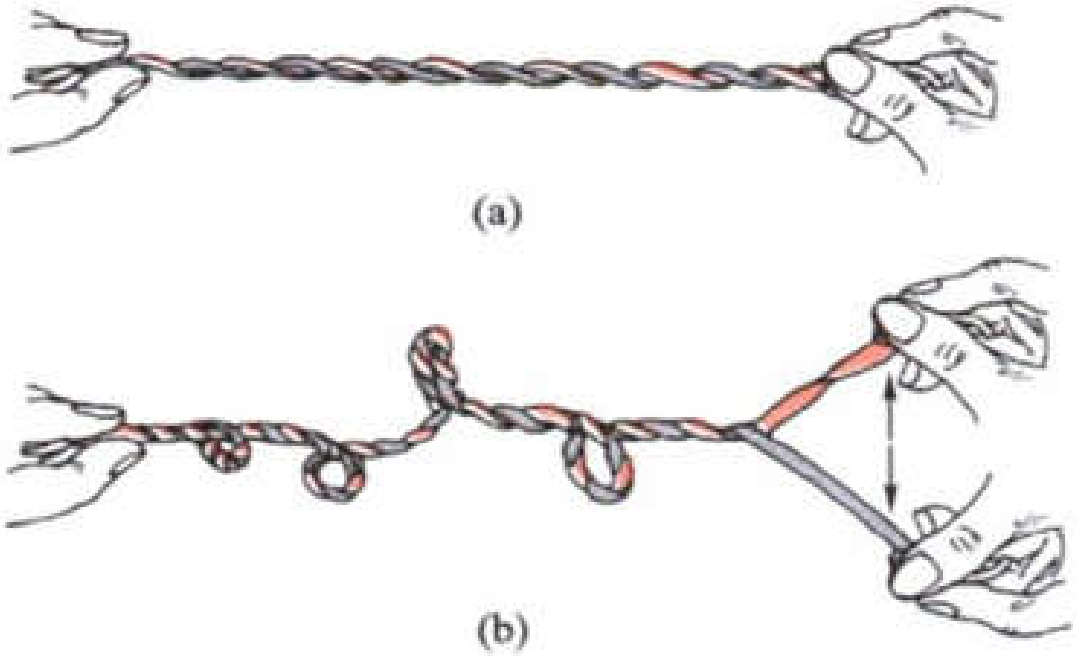
1.4.3 Single-stranded DNA binding proteins (SSBs)

SSBs aid helicase action by binding tightly and cooperatively to newly formed ssDNA and keeping it from annealing (退火) with its partner (renaturing, 复性). By coating the ssDNA, SSBs also protect it from degradation.

SSB single-strand binding protein (~60/fork)

Prokaryotes: SSB
Eukaryotes: RPA
(replication protein A)

1.4.4 Topoisomerase (拓扑异构酶)



- **Topoisomerase neutralizes the positive supercoils by introducing transient (瞬时的) single- or double-stranded breaks into DNA.**

Types of topoisomerases

(1) Type Ⅰ topoisomerases

- Introduce temporary single-stranded breaks in DNA.
- Not need ATP

(2) Type Ⅱ topoisomerases

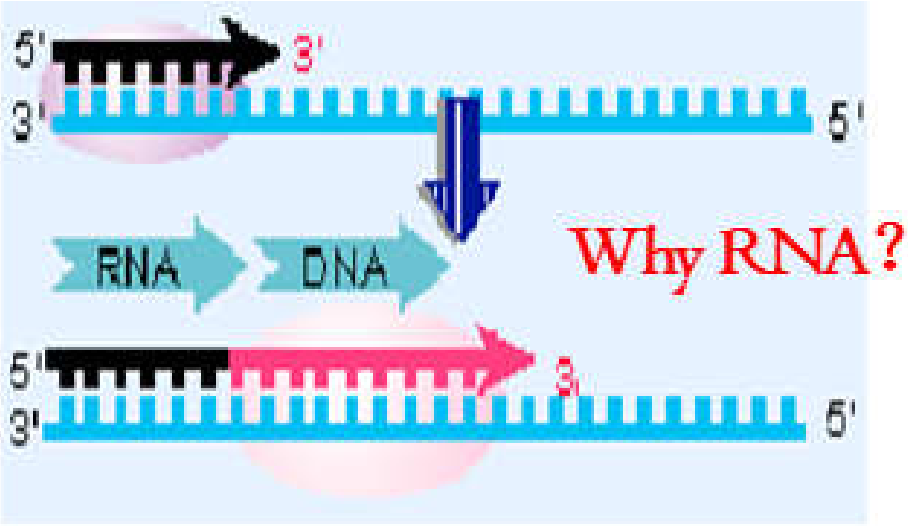
- Break and reseal (闭合) both DNA strands by hydrolysis of ATP.
- e.g. DNA gyrase 旋转酶(Topo Ⅱ), Topo Ⅳ
- Inhibitors of DNA gyrase, such as novobiocin (新生霉素) and oxolinic acid (恶喹酸), are effective inhibitors of bacterial replication and have antibiotic (抗生素) activity.

1.4.5 Primase (引发酶)

- DNA polymerase cannot initiate DNA synthesis *de novo* (从头合成).
- DNA synthesis is primed (引发) by RNA.

  10 ~12 nt

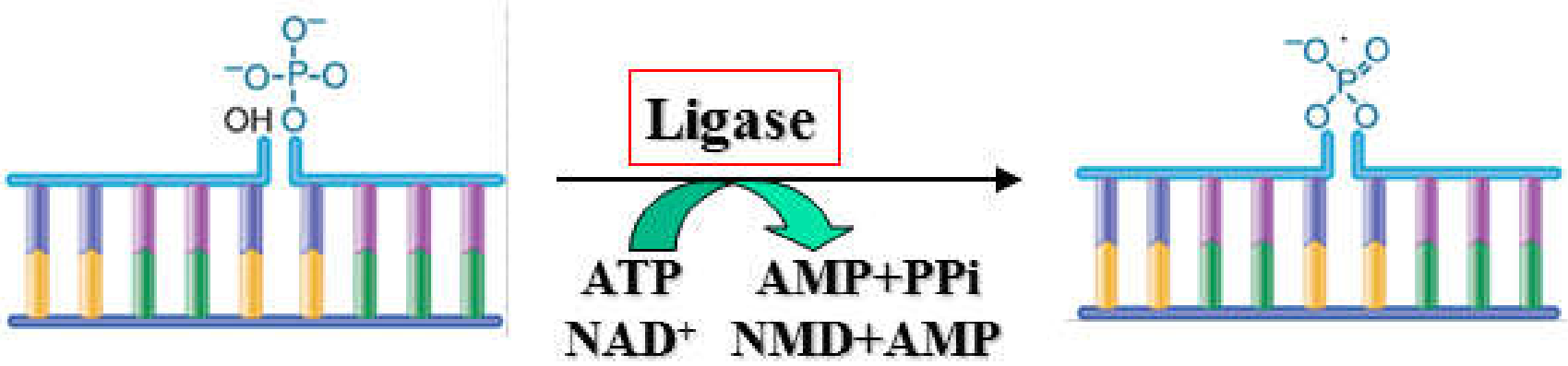
  fidelity (忠实性)



Primase
- RNA polymerase — M13 phage
- DnaG — *E. coli* and its other phages
- DNA Pol α — Eukaryotes

1.4.6 DNA ligase (连接酶)

DNA ligase makes the bond that connects the 3' end of one Okazaki fragment to the 5' beginning of the next fragment.



The ligase from *E. coli* uses the co-factor NAD^+ as energy source while ligases from Phage T4 and mammal cells use ATP.