DNA的复制
(DNA Replication)

Figure 6-1 Essential Cell Biology 3/e (© Garland Science 2010)

遗传信息忠实地从
一代传递到下一代

Key Terms

复制子(Replicon);

复制叉(Replication fork);

DNA的半保留复制 (Semi-conservative replication)

DNA的半不连续复制(semi-discontinuous replication)

冈崎片断(Okazaki fragment);

DNA聚合酶(DNA polymerase)

DNA的复制

- 生命的遗传是染色体DNA自我复制的结果;
- 染色体DNA的自我复制主要是通过半保留复制(Semi-conservative)来实现的，是一个以亲代DNA分子为模板合成子代DNA链的过程。

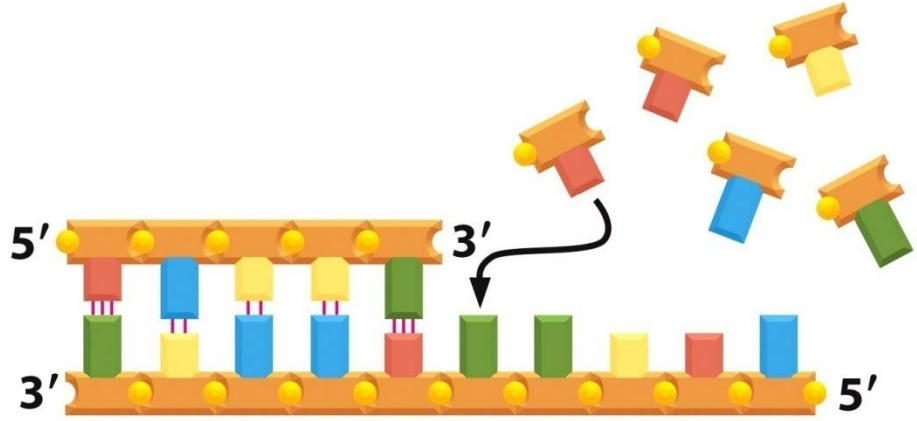


Figure 6-2 Essential Cell Biology 3/e (© Garland Science 2010)

DNA复制的三种预测模型

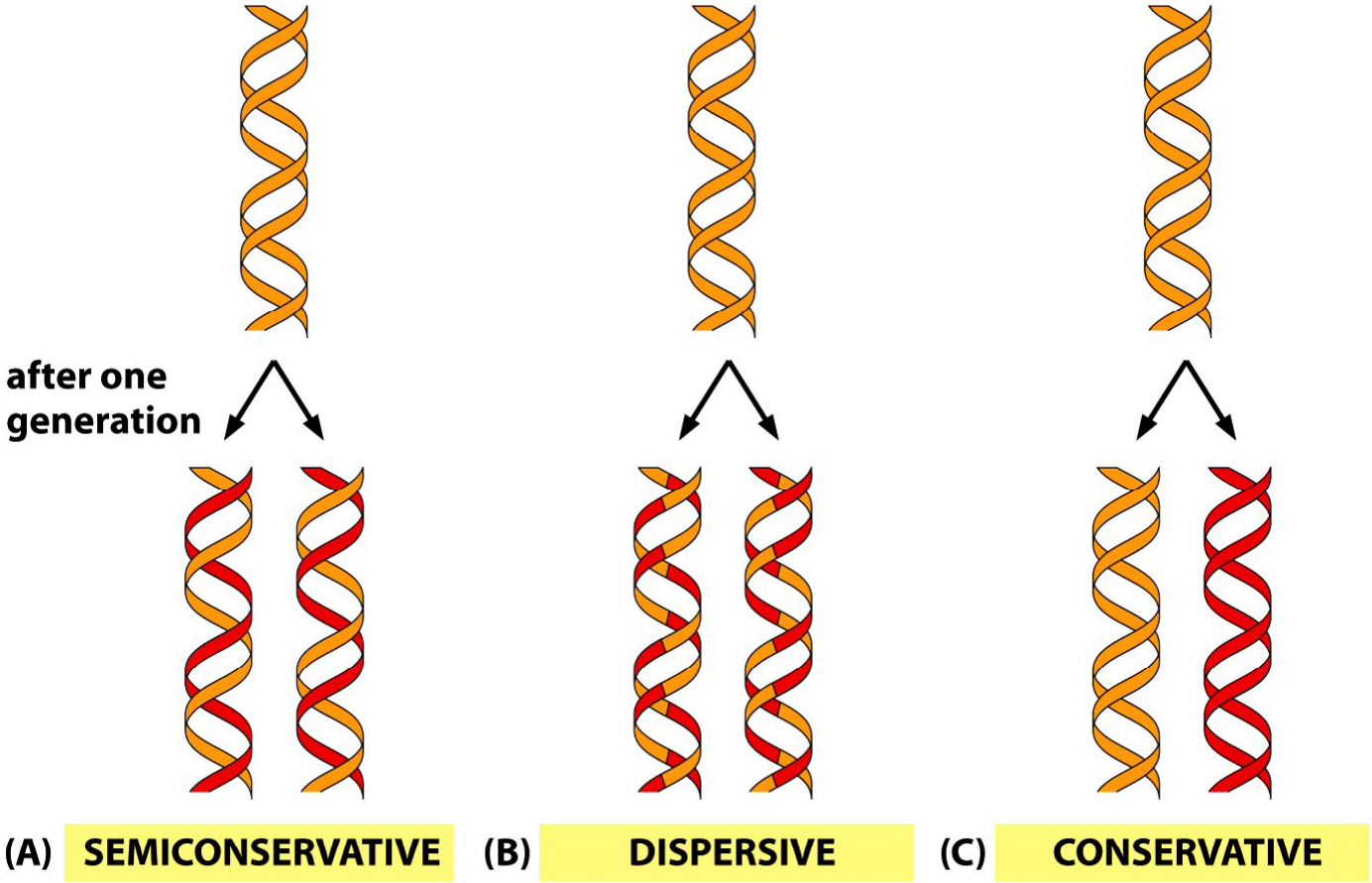


Figure 6-6 Essential Cell Biology 3/e (© Garland Science 2010)

证实DNA复制模型的实验

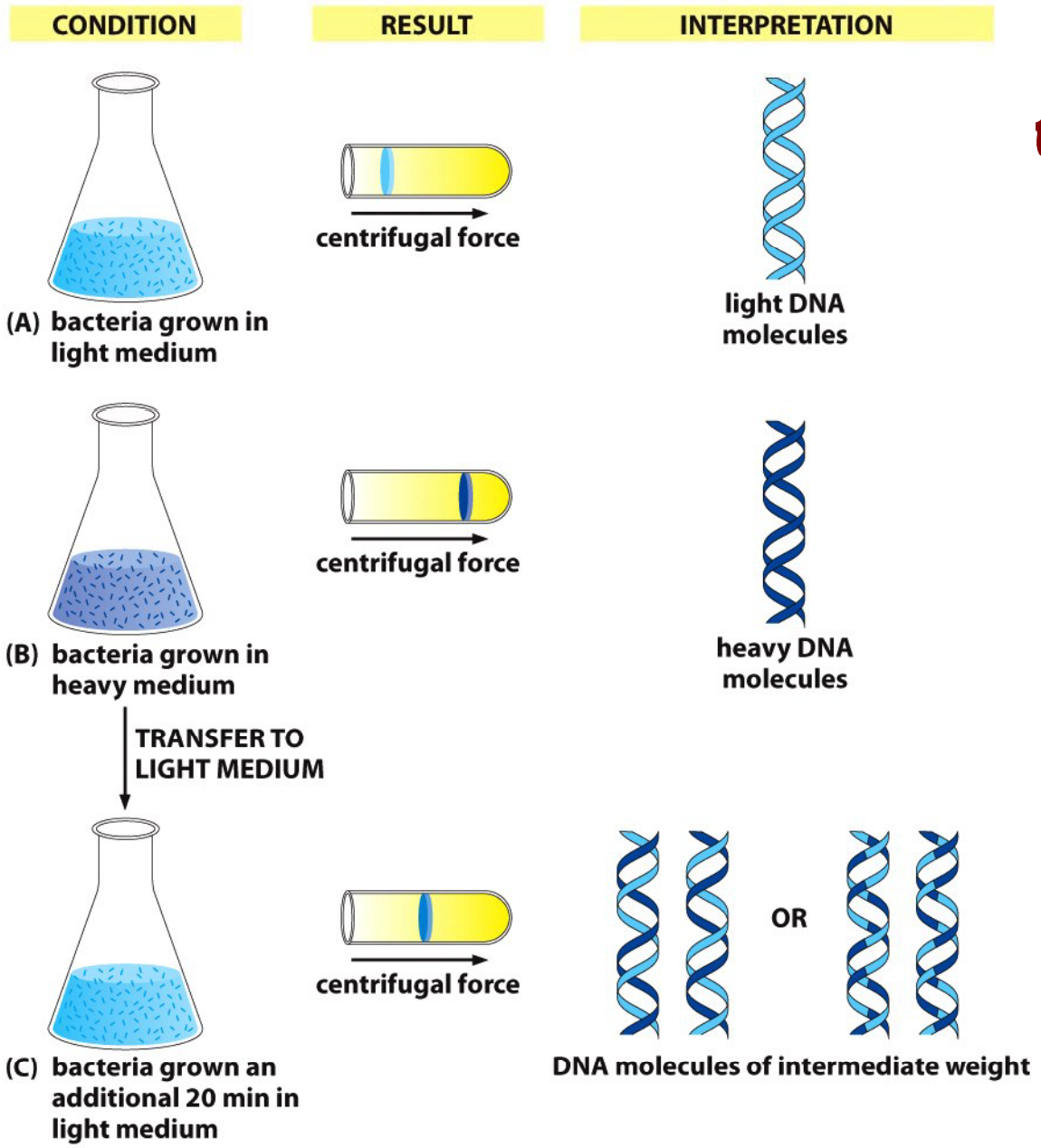
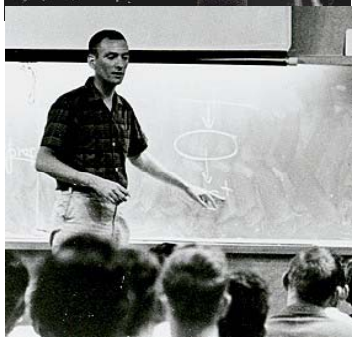
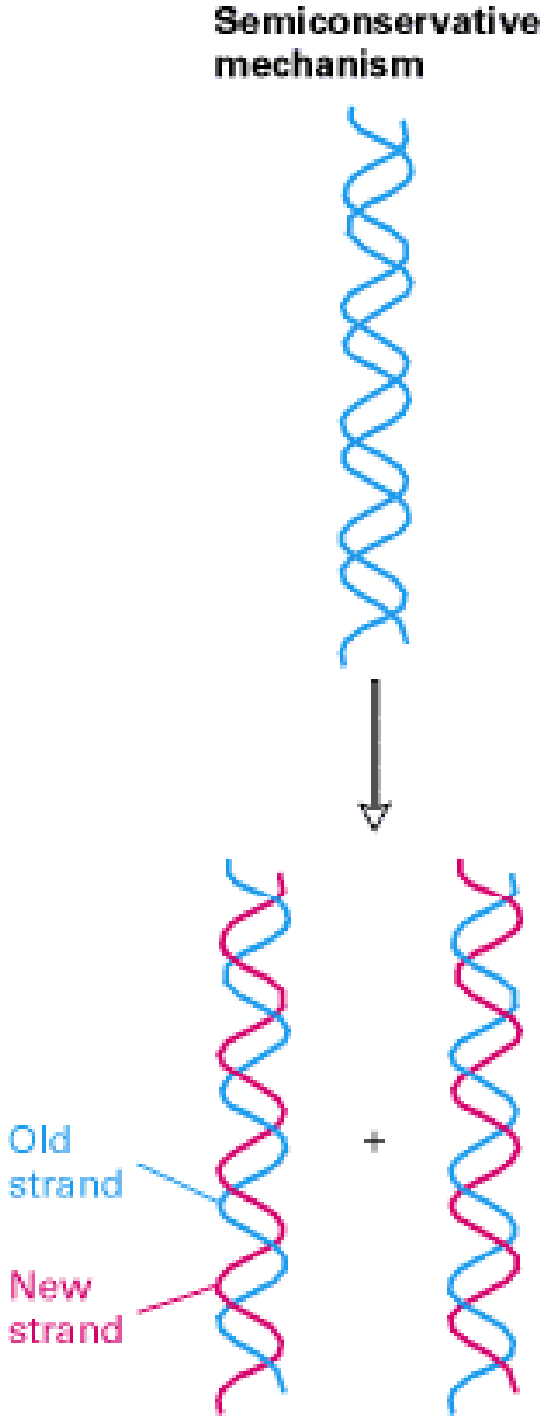


Figure 6-8 Essential Cell Biology 3/e (© Garland Science 2010)

Stahl



Meselson

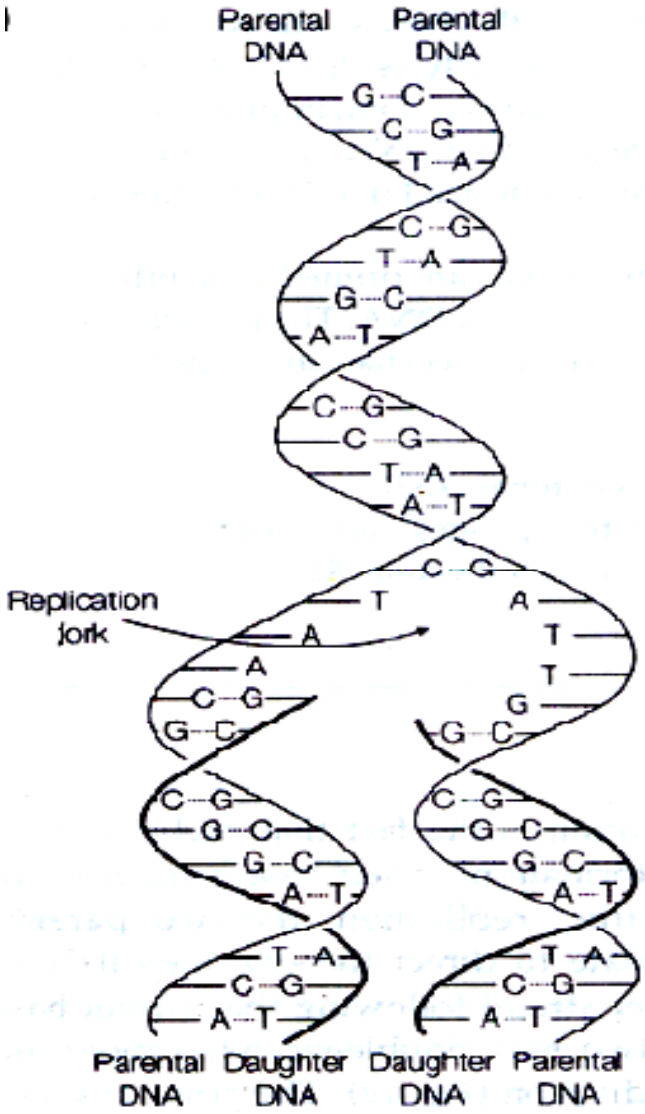


DNA的半保留复制
（semi-conservative replication）

DNA在复制过程中，每条链分别作为模板合成新链，产生互补的两条链。这样新形成的两个**DNA**分子与原来**DNA**分子的碱基顺序完全一样。因此，每个子代分子的一条链来自亲代**DNA**，另一条链则是新合成的，这种复制方式被称为**DNA**的半保留复制。

DNA的半保留复制保证了**DNA**在代谢上的稳定性，与**DNA**的遗传功能相符合。

复制叉



复制时，双链**DNA**要解开成两股链分别进行，所以，复制起点呈叉子形式，被称为复制叉**(Replication fork)**。

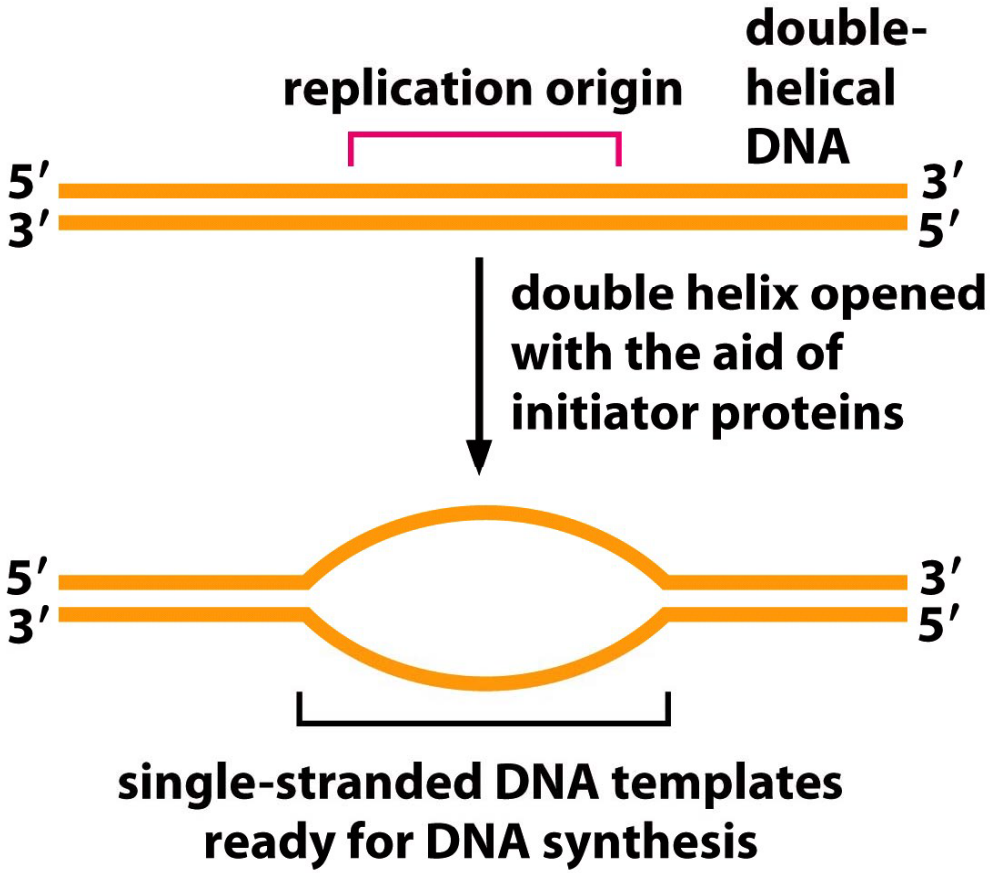


Figure 6-5 Essential Cell Biology 3/e (© Garland Science 2010)

复制子

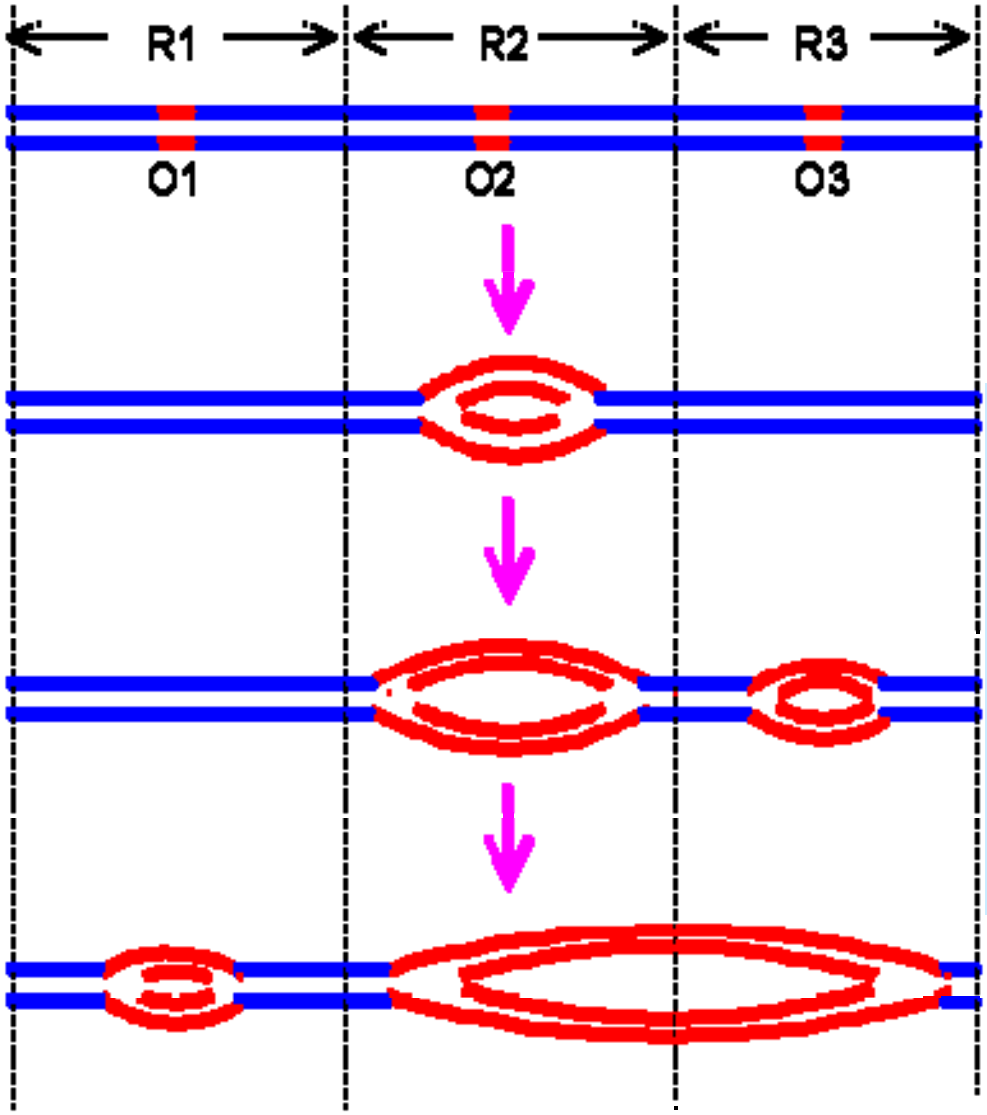
DNA的复制是由固定的起始点开始的。一般把生物体的复制单位称为**复制子(replicon)**。一个复制子只含一个复制起点。

部分生物复制子的比较

物种	细胞内复制子数目（个）	平均长度/kb	复制子移动速度 ($bp \cdot min^{-1}$)
大肠杆菌	1	4 200	50 000
酵母	500	40	3 600
果蝇	3 500	40	2 600
蟾蜍	15 000	200	500
蚕豆	35 000	300	?

- 细菌、病毒和线粒体的**DNA**分子都是作为单个复制子完成复制的；
- 真核生物基因组可以同时在多个复制起点上进行复制，也就是说它们的基因组包含有多个复制子。

Multiple eukaryotic replicons



O1, O2, and O3 are replication origins, each serving a region called replicon (R1, R2, and R3).

DNA replication involves unwinding of the double helix. Unwinding of a DNA molecule looks like a "fork" growing in one direction.
The region being replicated looks like a bubble called the "replication bubble" (in red).

Electron Microscopy of replicating DNA reveals replicating bubbles

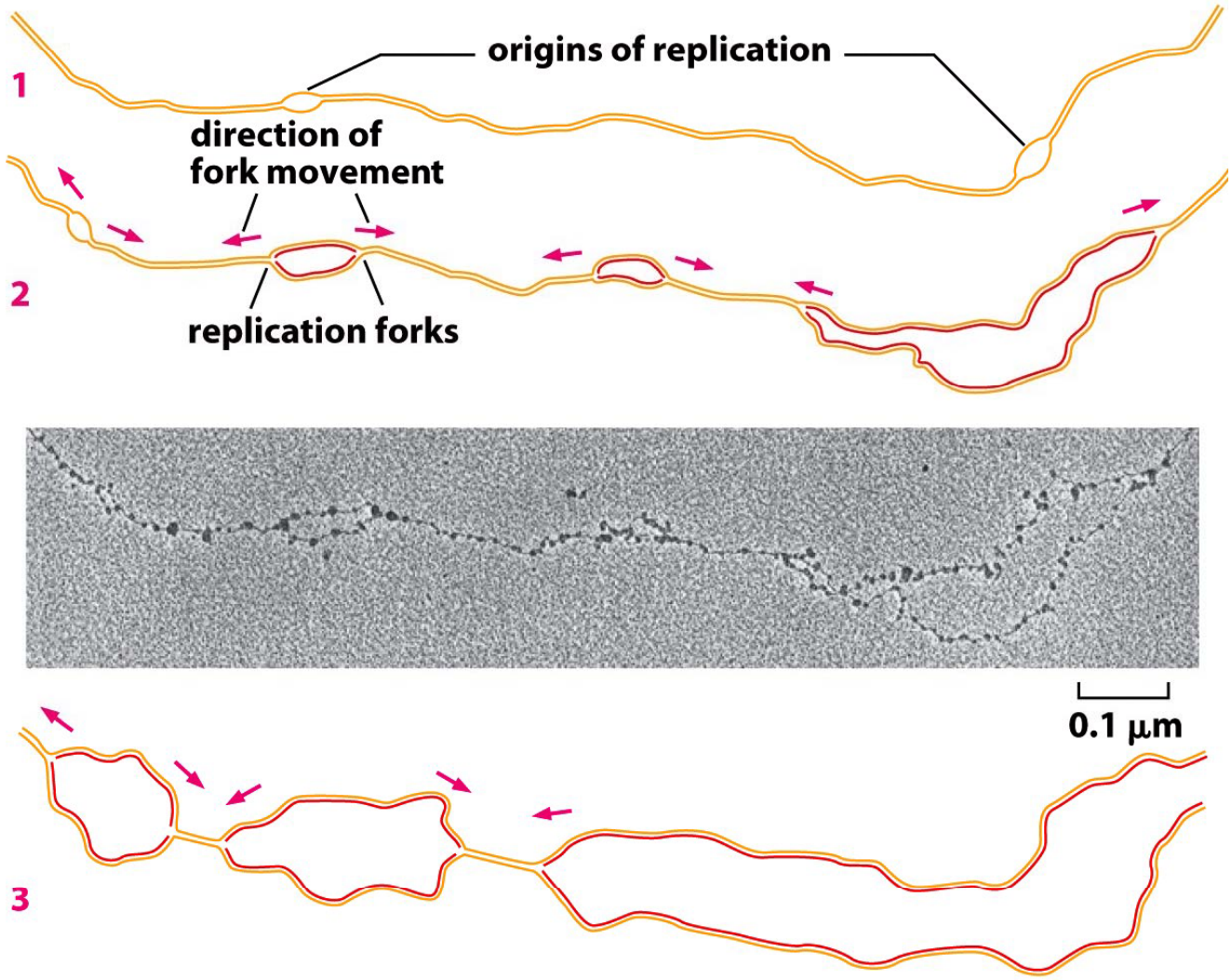


Figure 6-9 Essential Cell Biology 3/e (© Garland Science 2010)

早期果蝇胚胎中正在复制的DNA

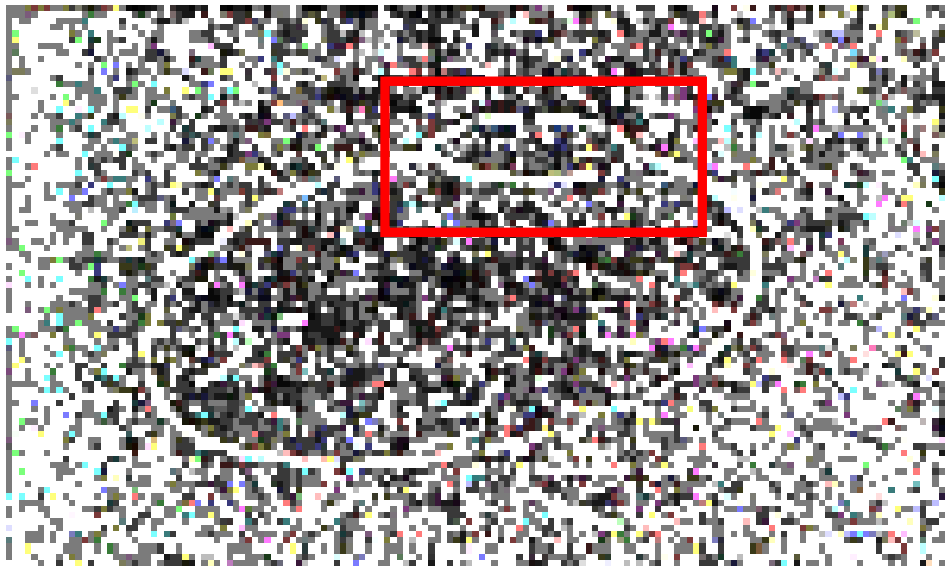
原核生物DNA的复制

Replication of the *E. coli* chromosome

In 1963, John Cairns' *Technique*:

- Grew *E. coli* in ^{3}H thymidine
- Waited till cells were in the middle of replication
- Lysed the cells very very gently
- Spread the lysate on an EM grid
- Exposed the grid to X-ray film for TWO months.

What Cairns' experiment showed:



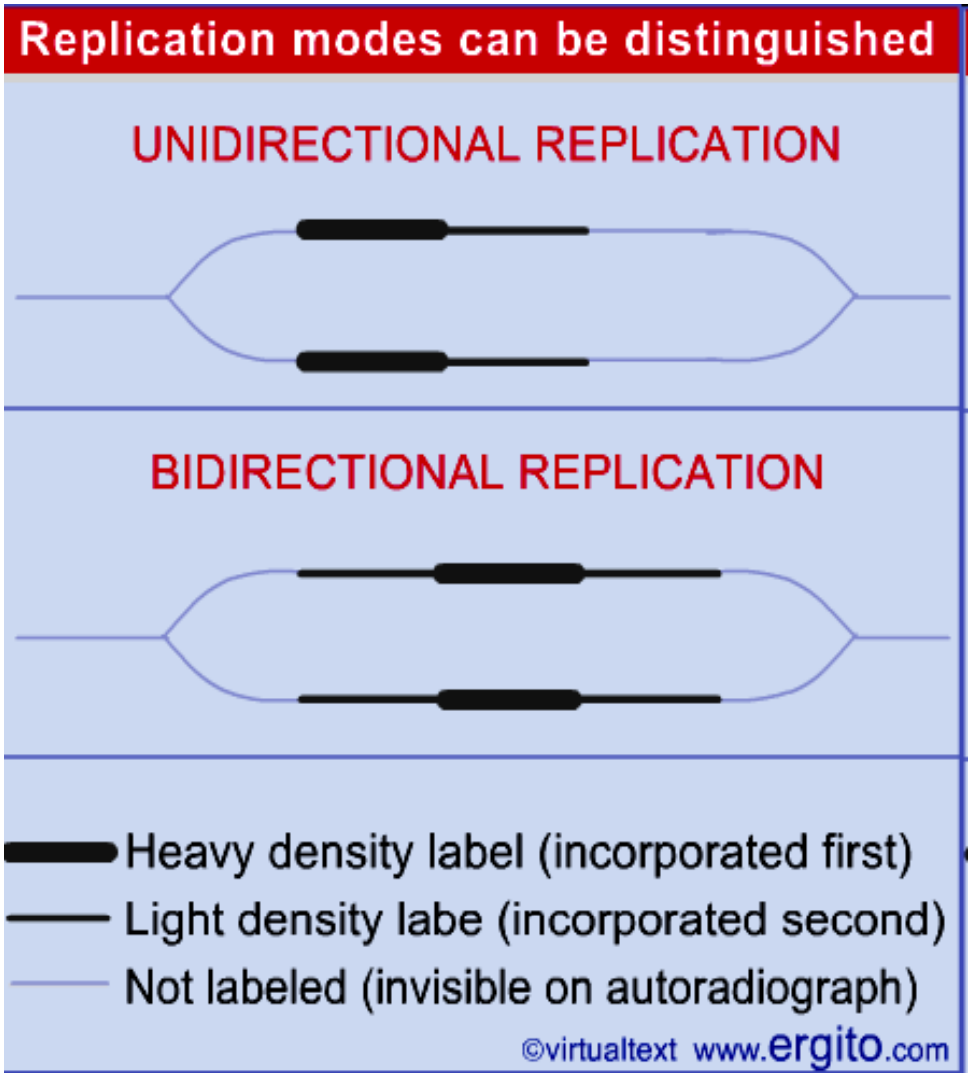
Theta Forms

θ

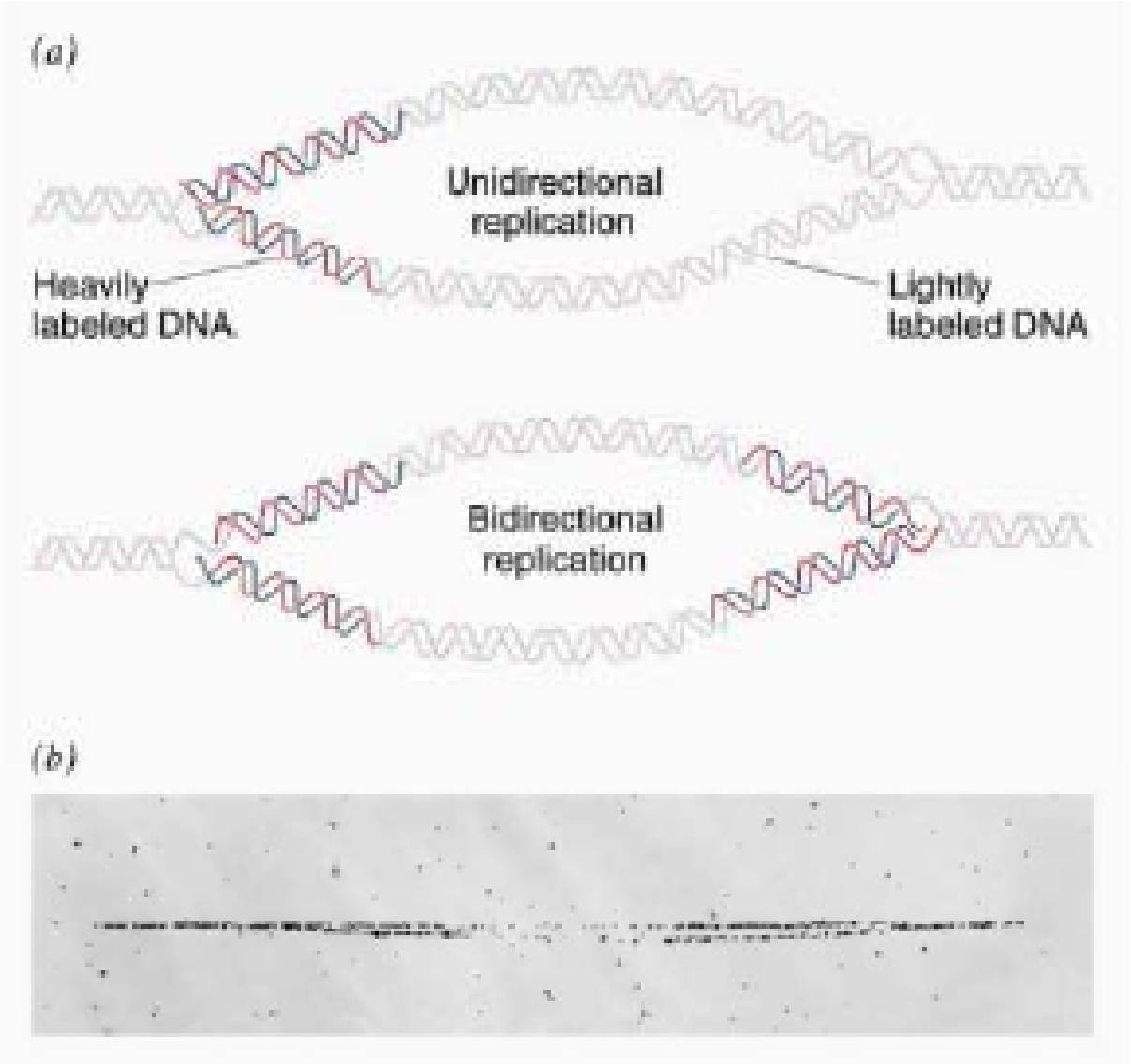
- *E. coli* has a circular chromosome.
- *E. coli* has a single origin of replication.
- In *E. coli*, replication and unwinding are simultaneous.

What Cairns did not show:

- Is replication UNIdirectional or BIdirectional?

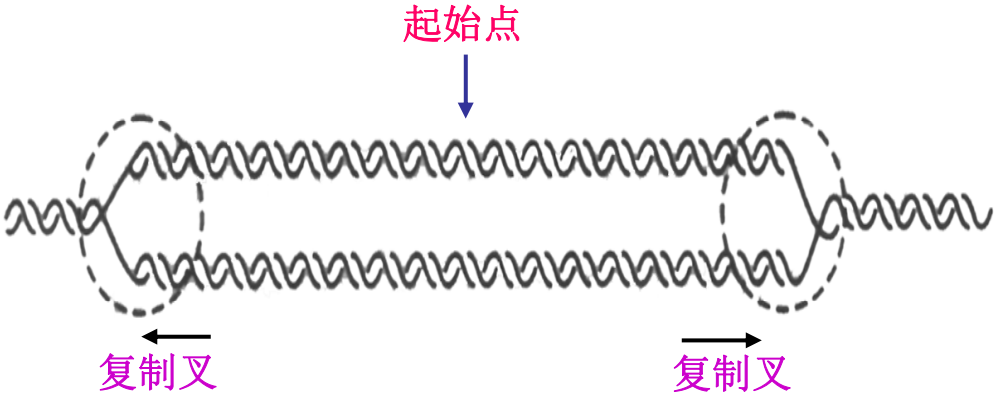


Bidirectional Replication
(双向复制)

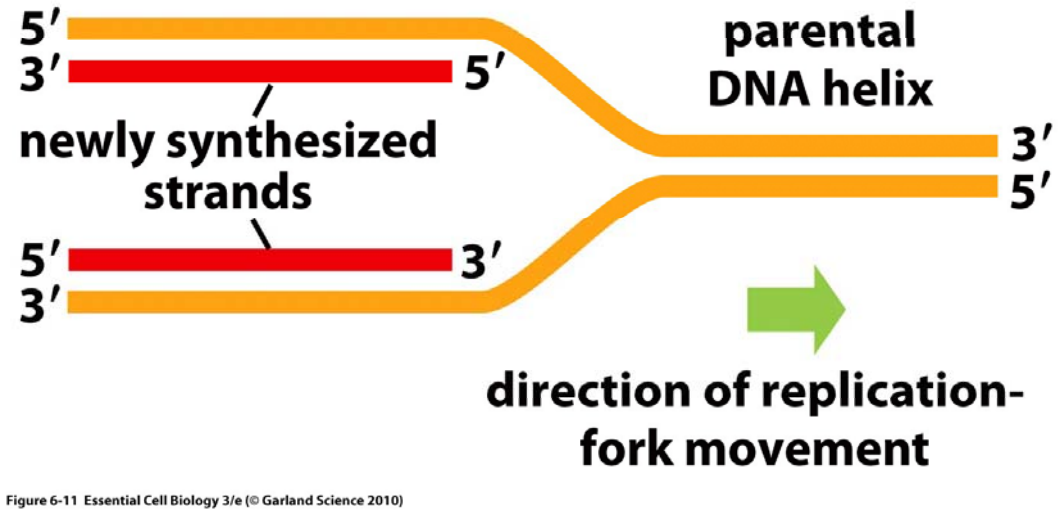


Bidirectional Replication
(双向复制)

- 无论是原核生物还是真核生物，**DNA**的复制主要是从固定的起始点以双向等速复制方式进行的。
- 复制叉以**DNA**分子上某一特定顺序为起点，向两个方向等速生长前进。



DNA Synthesis



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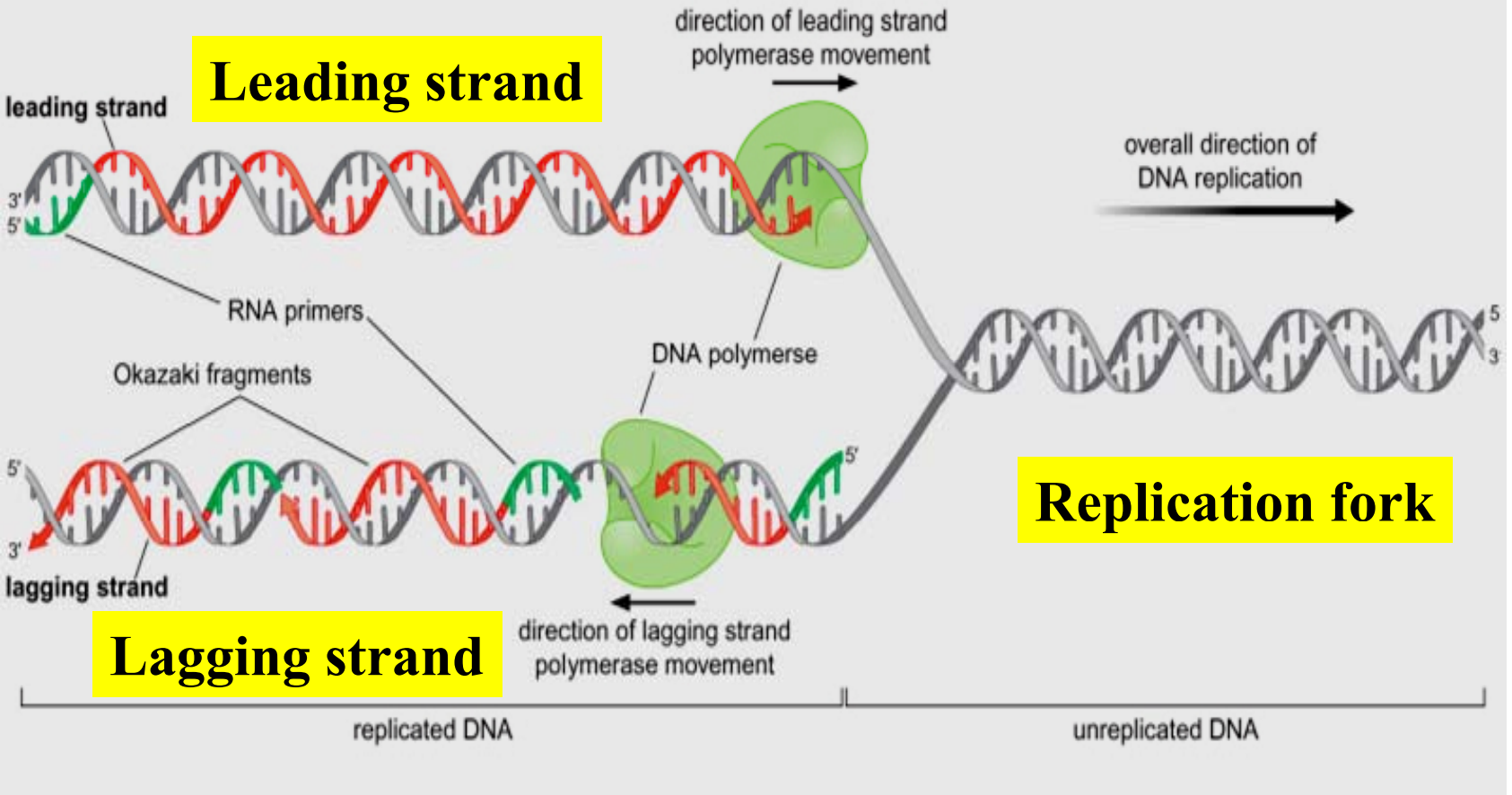
- 由于**DNA**双螺旋的两条链是反向平行的，因此两个模板极性不同。
- 所有已知**DNA**聚合酶的合成方向都是**5’→3’**

为了解释DNA的等速复制现象，日本学者冈崎(Okazaki)等提出了DNA的半不连续复制模型(semi-discontinuous replication)。

半不连续复制 Semi-discontinuous replication

冈崎片段(Okazaki fragment)



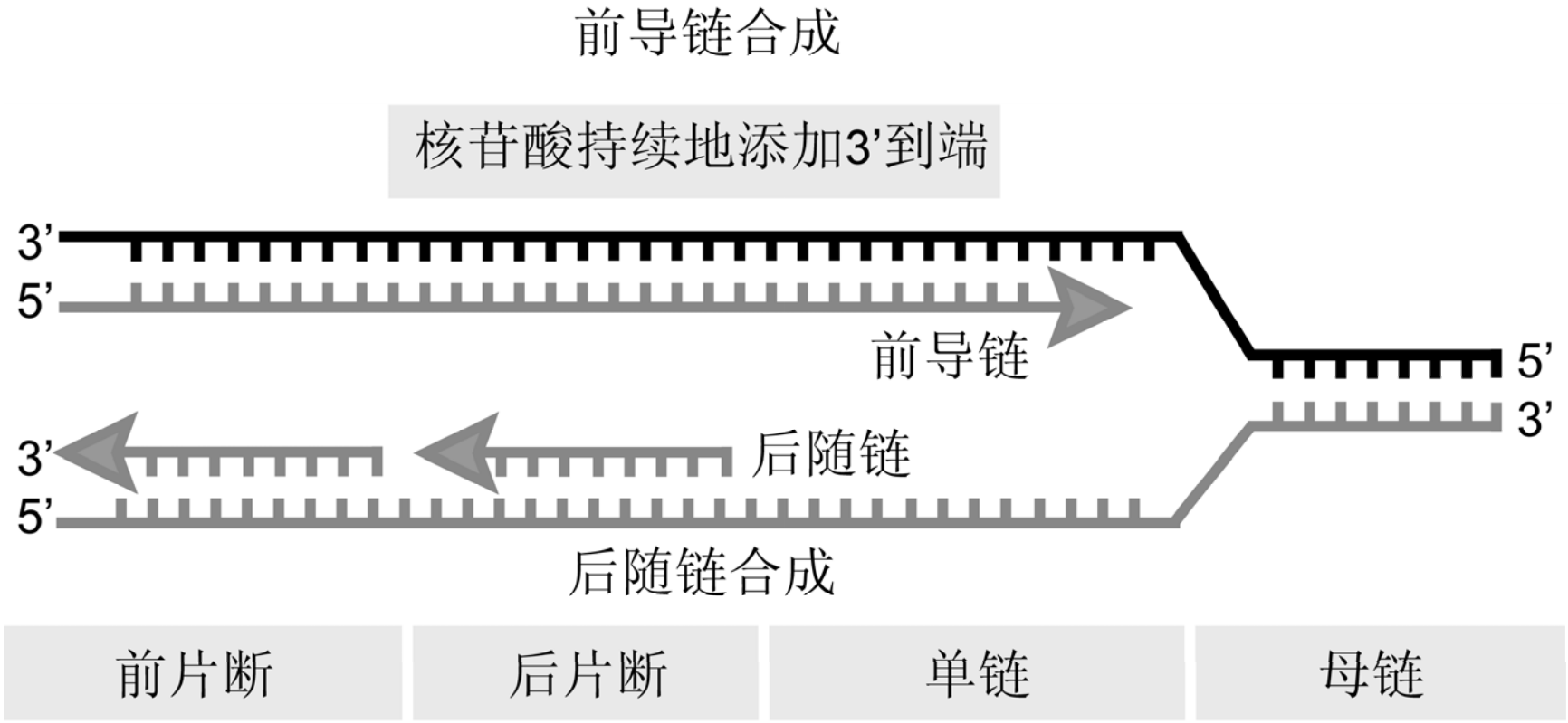
前导链的连续复制和滞后链的不连续复制在生物界是有普遍性的，因而称之为DNA的半不连续复制。

[^{3}H] Thymidine pulse-chase labeling and alkaline sucrose gradient: discovery of semi-discontinous replication

1、用^{3}H脱氧胸苷短时间标记后提取DNA，得到不少平均长度为2-3kb DNA片段。

2、用DNA连接酶温度敏感突变株进行实验，在连接酶不起作用的温度下，有大量小片段累积，说明复制过程中至少有一条链首先合成较短的片段，然后再生成大分子DNA。

DNA的半不连续复制
（semi-discontinuous replication）



·The short discontinuous segments are called Okazaki Fragments.

·In bacteria they are approximately 1000 nt in length; in eukaryotes they are approximately 200 nt in length.

DNA 复制的体系

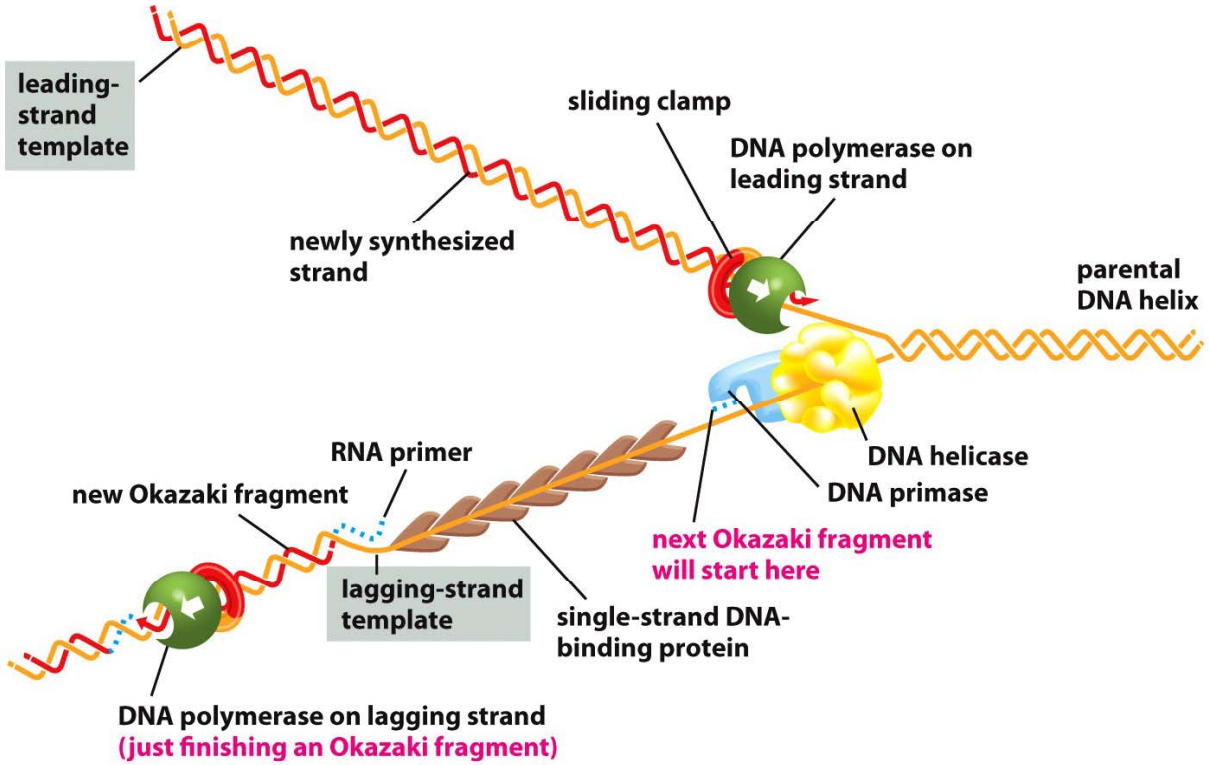


Figure 6-17a Essential Cell Biology 3/e (© Garland Science 2010)

- 亲代**DNA**分子为模板
- 四种脱氧三磷酸核苷**(dNTP)**为底物
- 提供**3'-OH**末端的引物
- 多种酶及蛋白质

DNA拓扑异构酶、**DNA**解链酶、单链结合蛋白、引物酶、**DNA**聚合酶、**RNA**酶以及**DNA**连接酶等

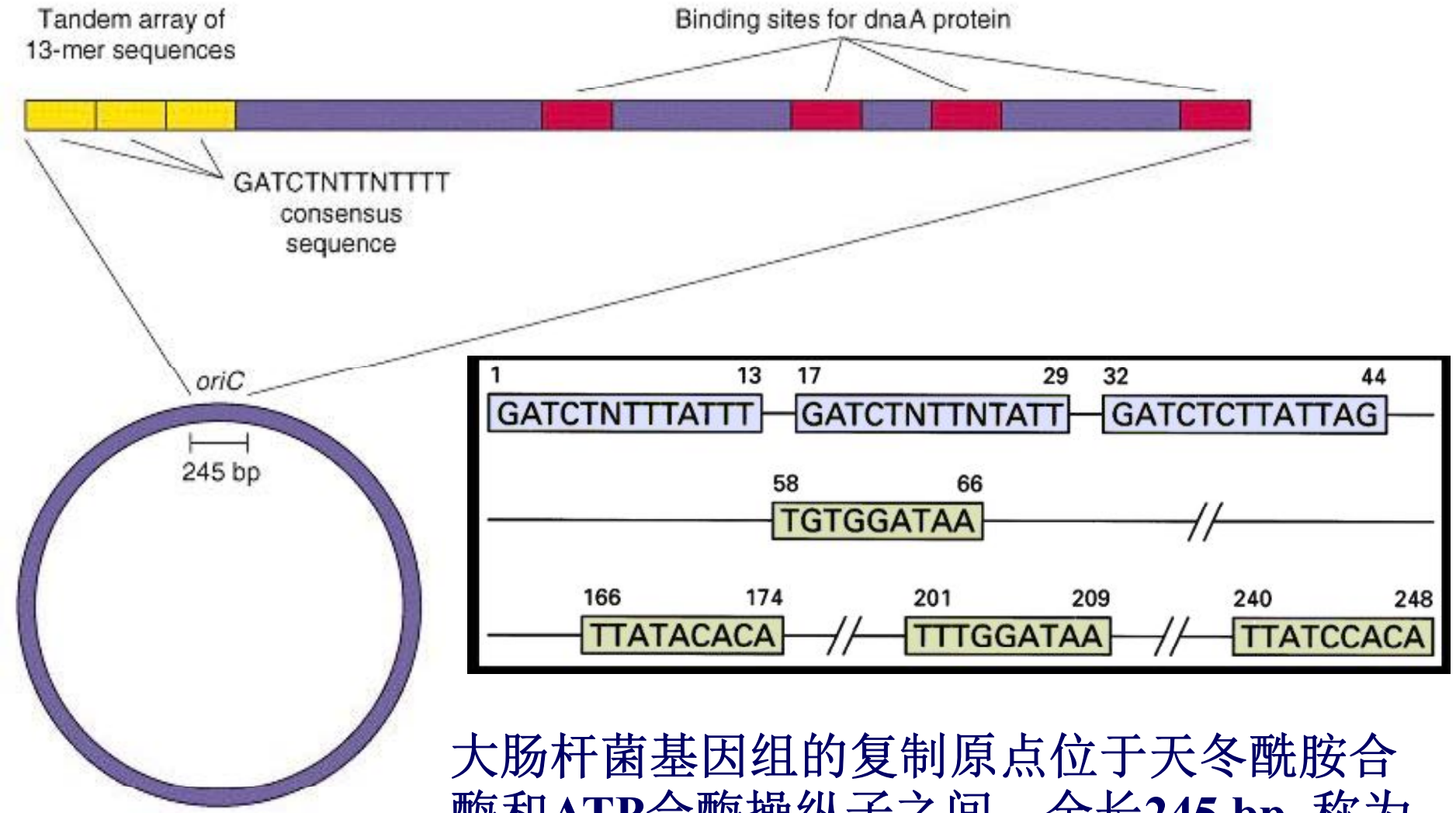
DNA复制的基本过程

- 复制的起始 (initiation)
- DNA链的延伸 (elongation)
- 复制的终止 (termination)

DNA复制的起始

- 复制起始原点
- **DNA**双螺旋的解旋
- 复制的引发

大肠杆菌(*E. coli*)的 *OriC* 复制原点

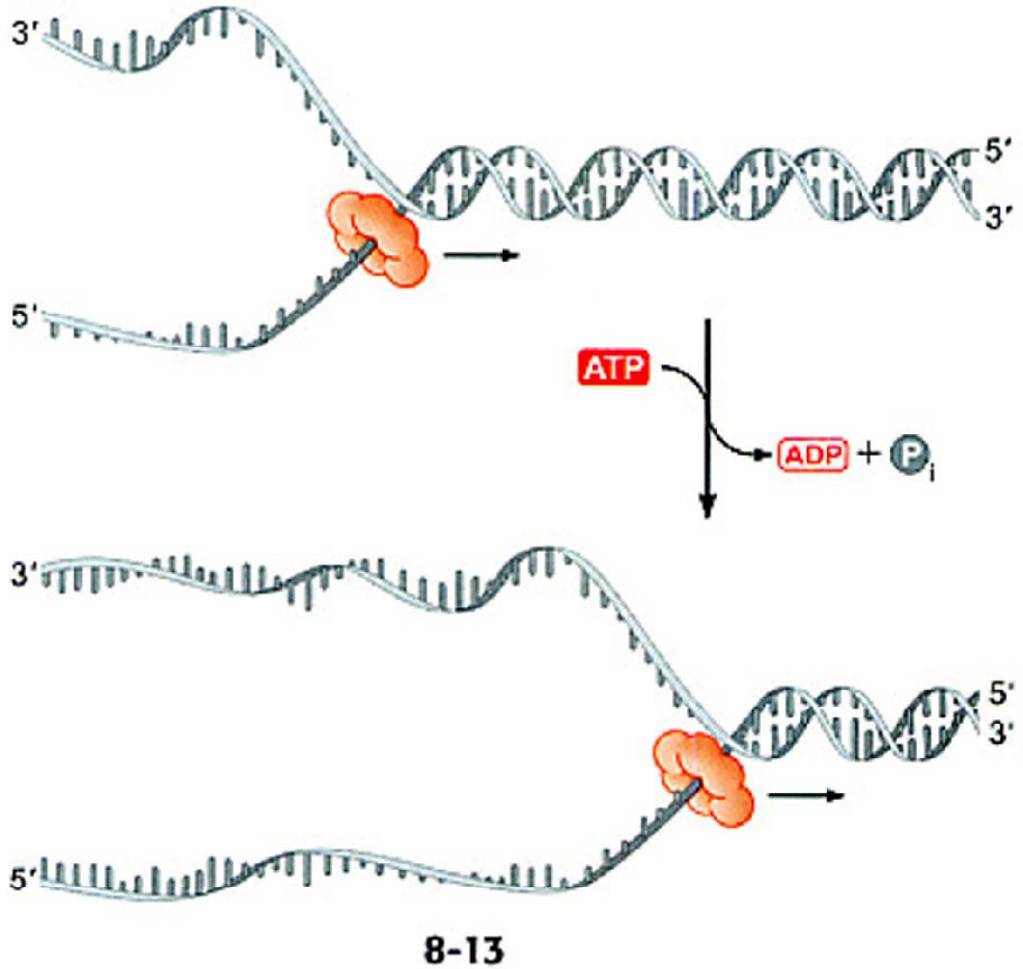


大肠杆菌基因组的复制原点位于天冬酰胺合酶和**ATP**合酶操纵子之间，全长**245 bp**, 称为***oriC***。

参与DNA复制起始和引发的蛋白质

- **DNA解旋酶(DNA helicase)**
  催化**DNA**双链的解链过程。
- 单链**DNA**结合蛋白**(single strand DNA binding protein)**：
  以四聚体形式存在于复制叉处，只保持单链的存在，并不能起解链作用。
- **DNA**拓扑异构酶**(DNA topoisomerase)**
  消除**DNA**双链的超螺旋堆积。
- 引物酶**(primase)**
  合成一小段**RNA**引物，为**DNA**新链的合成提供**3'-OH**末端。

DNA双螺旋的解旋



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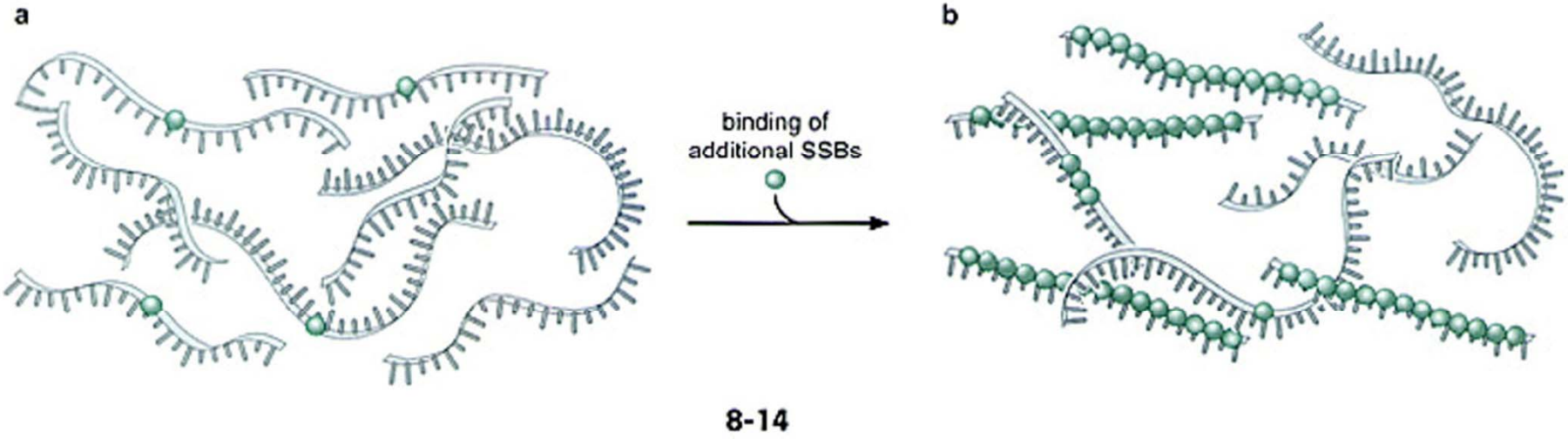
DNA helicases separate the two strands of the double helix.

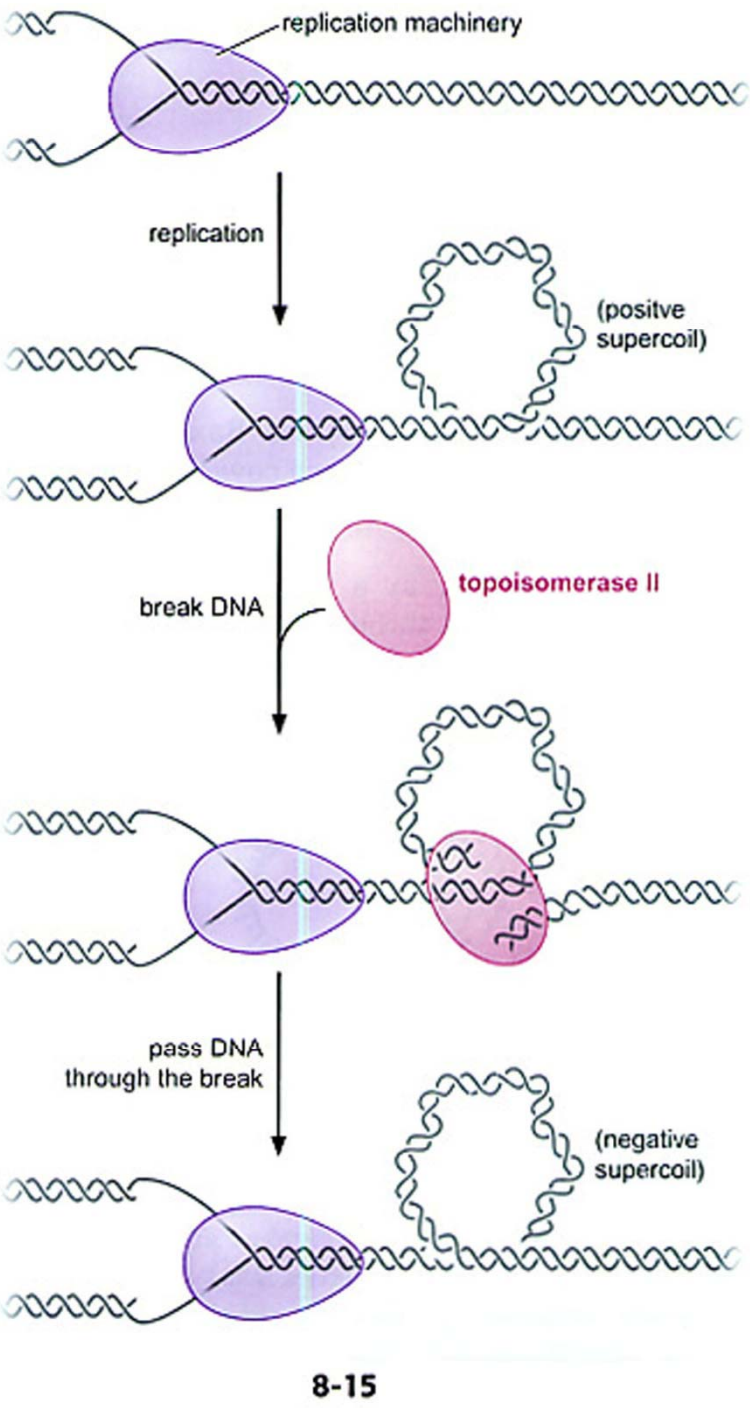
DNA双螺旋的解旋



Binding of SSB to DNA inhibits the formation of intramolecular base pairs.

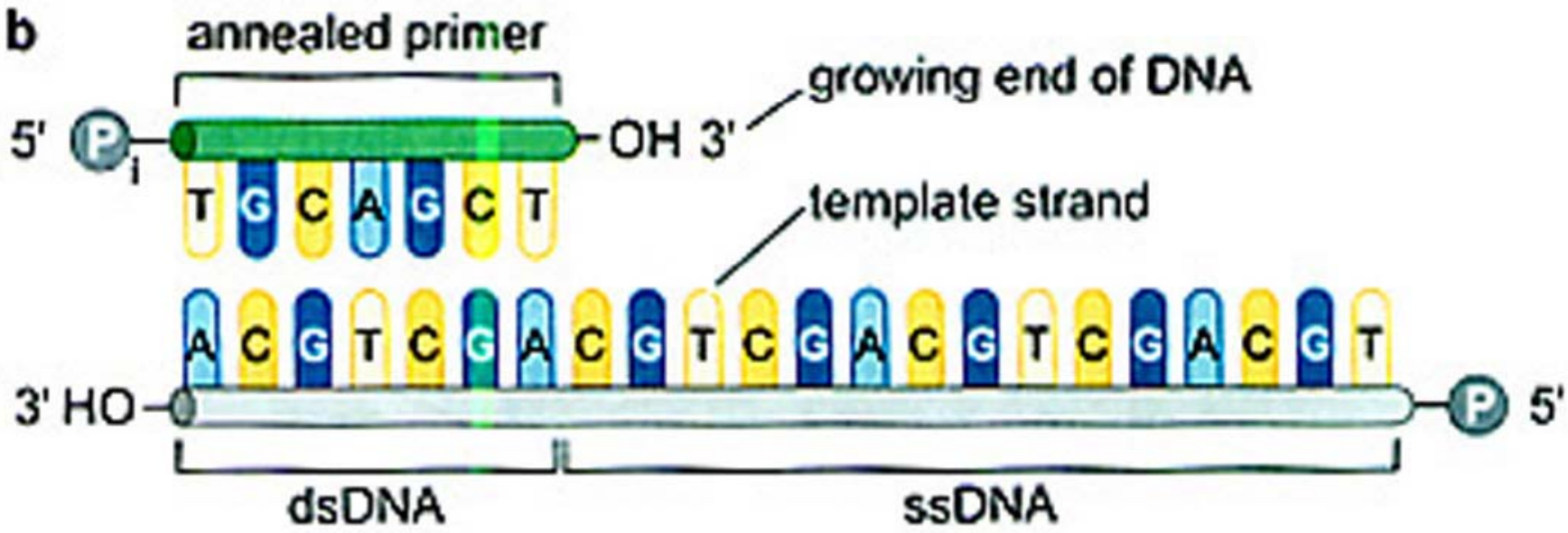
Action of topoisomerases at the replication fork

Topoisomerase rapidly remove the positive supercoils accumulate in front of the replication fork.



DNA聚合酶只能延长已存在的DNA链，而不能从头合成DNA链，那么，新DNA的复制是怎样开始的呢？

DNA polymerase requires a 3'-OH end to initiate replication

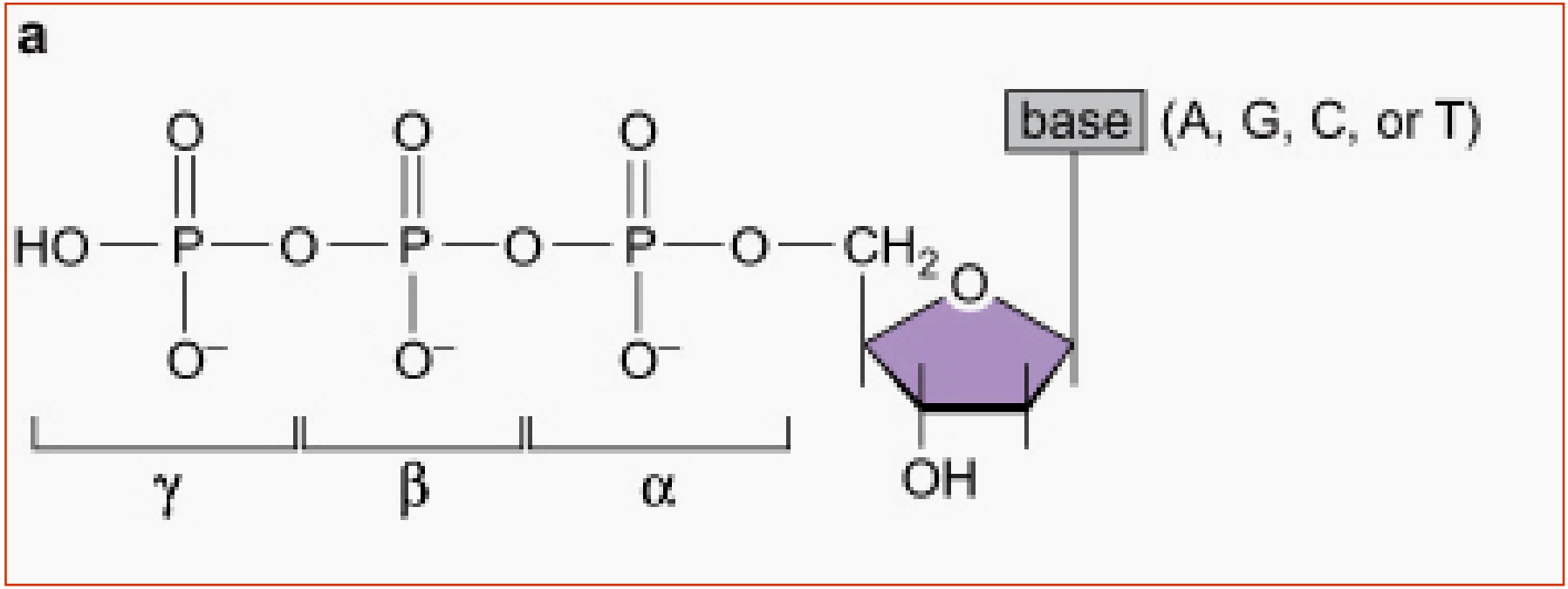


The 3'-OH end is called a **primer**.

A **primer** is a short sequence (often of RNA) that is paired with one strand of DNA and provides a free 3' -OH end at which a DNA polymerase starts synthesis of a deoxyribonucleotide chain.

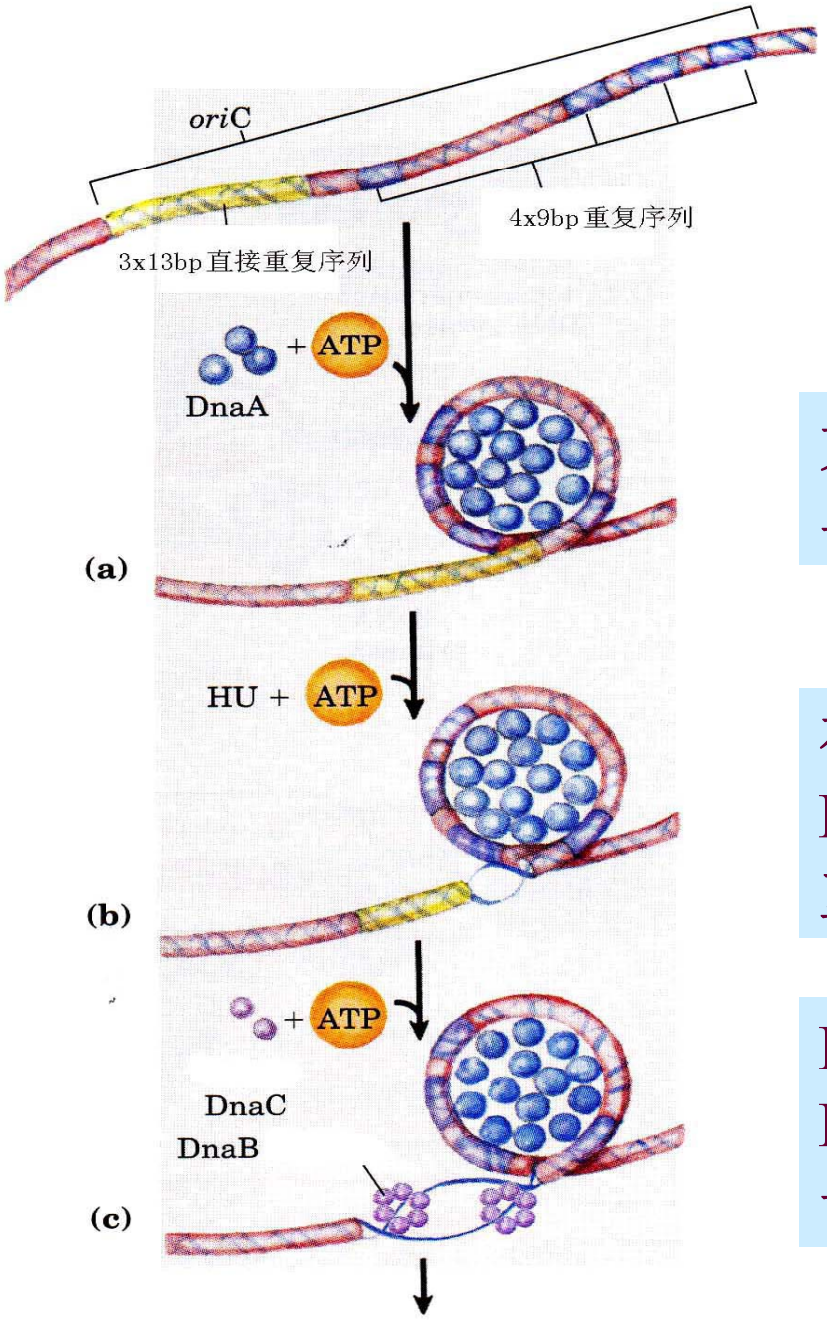
The **primase** is a type of RNA polymerase that synthesizes short segments of RNA that will be used as primers for DNA replication.

Substrates required for DNA synthesis



Both a primer and a template are essential for all DNA synthesis

由大肠杆菌oriC复制起始点处引发的DNA复制过程



大约20个DnaA蛋白在ATP的作用下与oriC处的4个9 bp保守序列相结合。

↓

在Hu蛋白和ATP的共同作用下，DnaA复制起始复合物使3x13 bp直接重复序列变形，形成开链。

↓

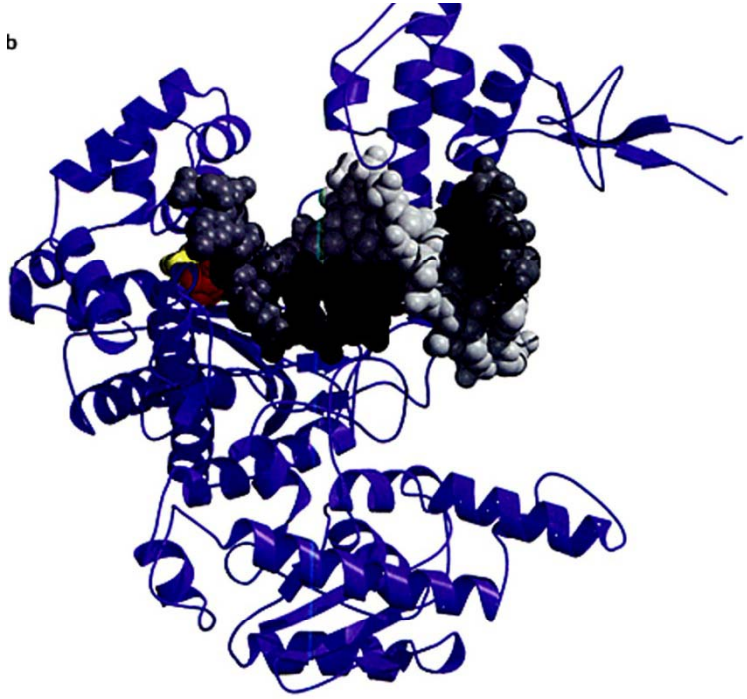
DnaB（解链酶）六体分别与单链DNA相结合（需要DnaC的帮助）进一步解开DNA双链。

↓

与引物结合，起始DNA复制。

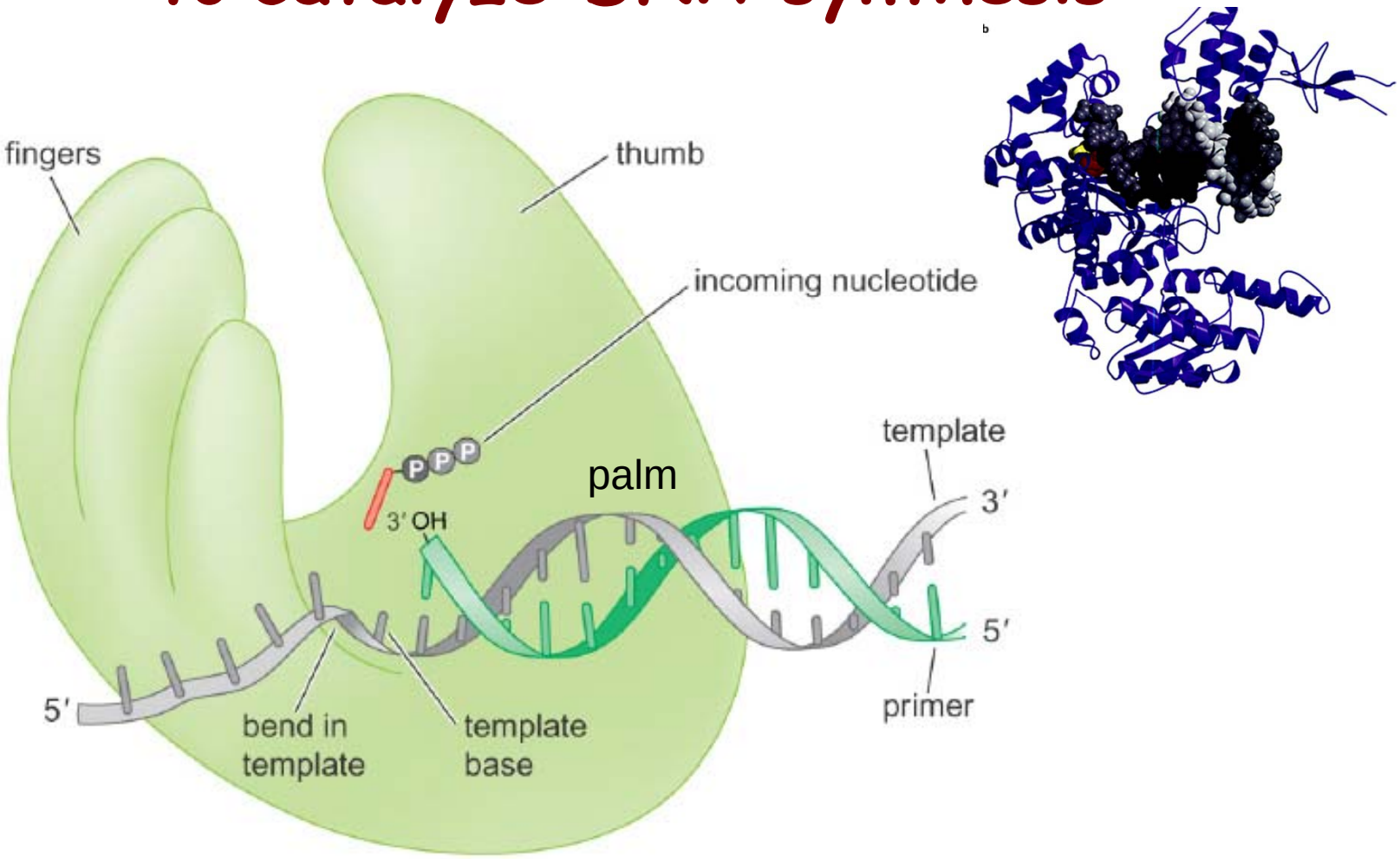
DNA链的延伸

DNA链的延伸需要的蛋白质:



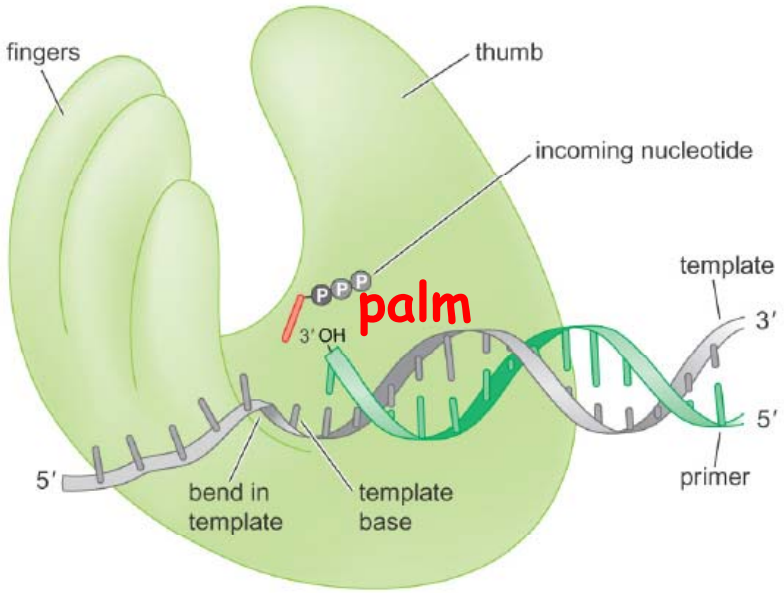
- **DNA聚合酶**
- 滑动夹（**Sliding DNA clamp**）
- **RNA酶(RNase H 等)**
  在复制完成后切除**RNA**引物。
- **DNA连接酶 (DNA ligase)**
  通过生成**3'5'-**磷酸二酯键连接两条**DNA**链。

DNA polymerase use a single active site to catalyze DNA synthesis



DNA polymerase bound to a primer:template junction

DNA polymerase



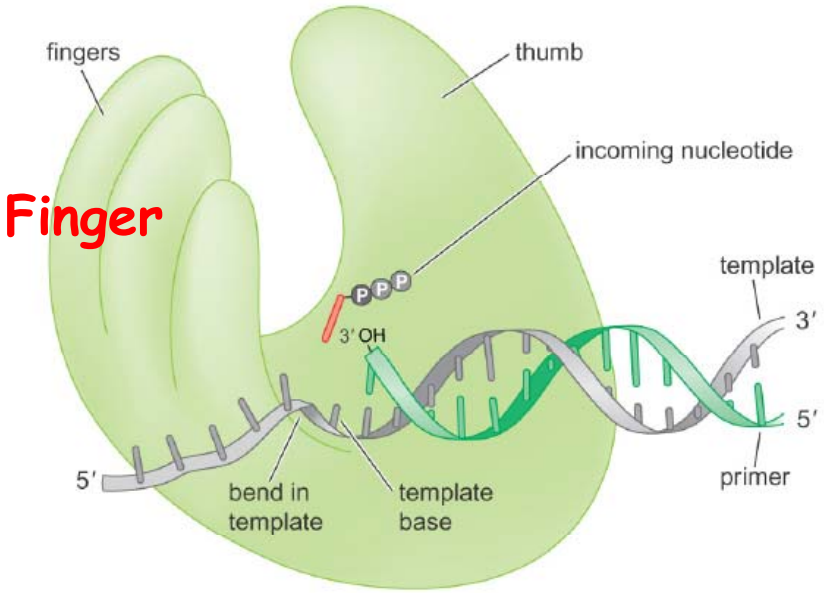
palm domain:

1. Contains two catalytic sites, one for addition of dNTPs and one for removal of the mispaired dNTP.

2. The polymerization site binds to two metal ions that alter the chemical environment around the catalytic site and lead to the catalysis.

3. Monitors the accuracy of base-pairing for the most recently added nucleotides by forming extensive hydrogen bond contacts with minor groove of the newly synthesized DNA.

DNA polymerase

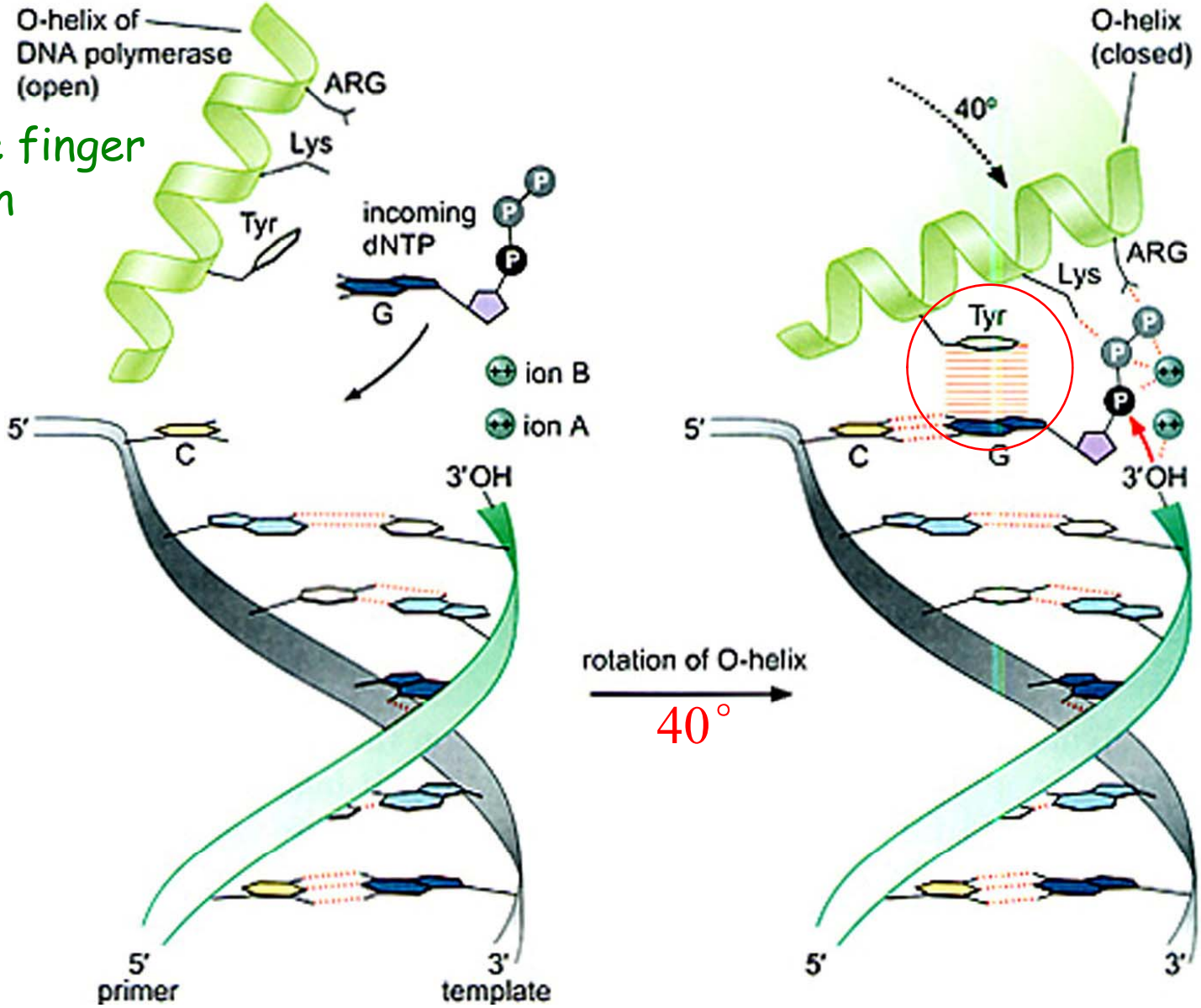


Finger domain:

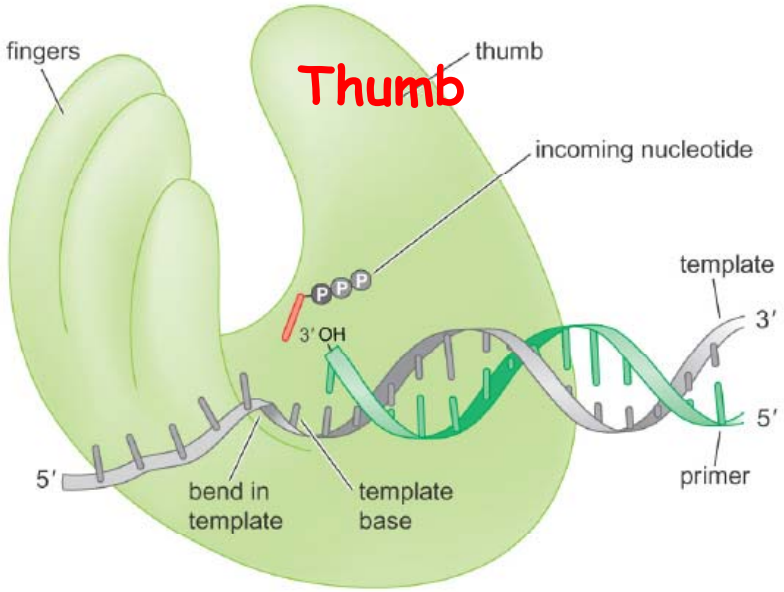
- Binds to the incoming dNTP, encloses the correct paired dNTP to the position for catalysis
- Bends the template to expose the only nucleotide at the template that ready for forming base pair with the incoming nucleotide

DNA polymerase "grips" the template and the incoming nucleotide when a correct base pair is made

In the finger domain



DNA polymerase



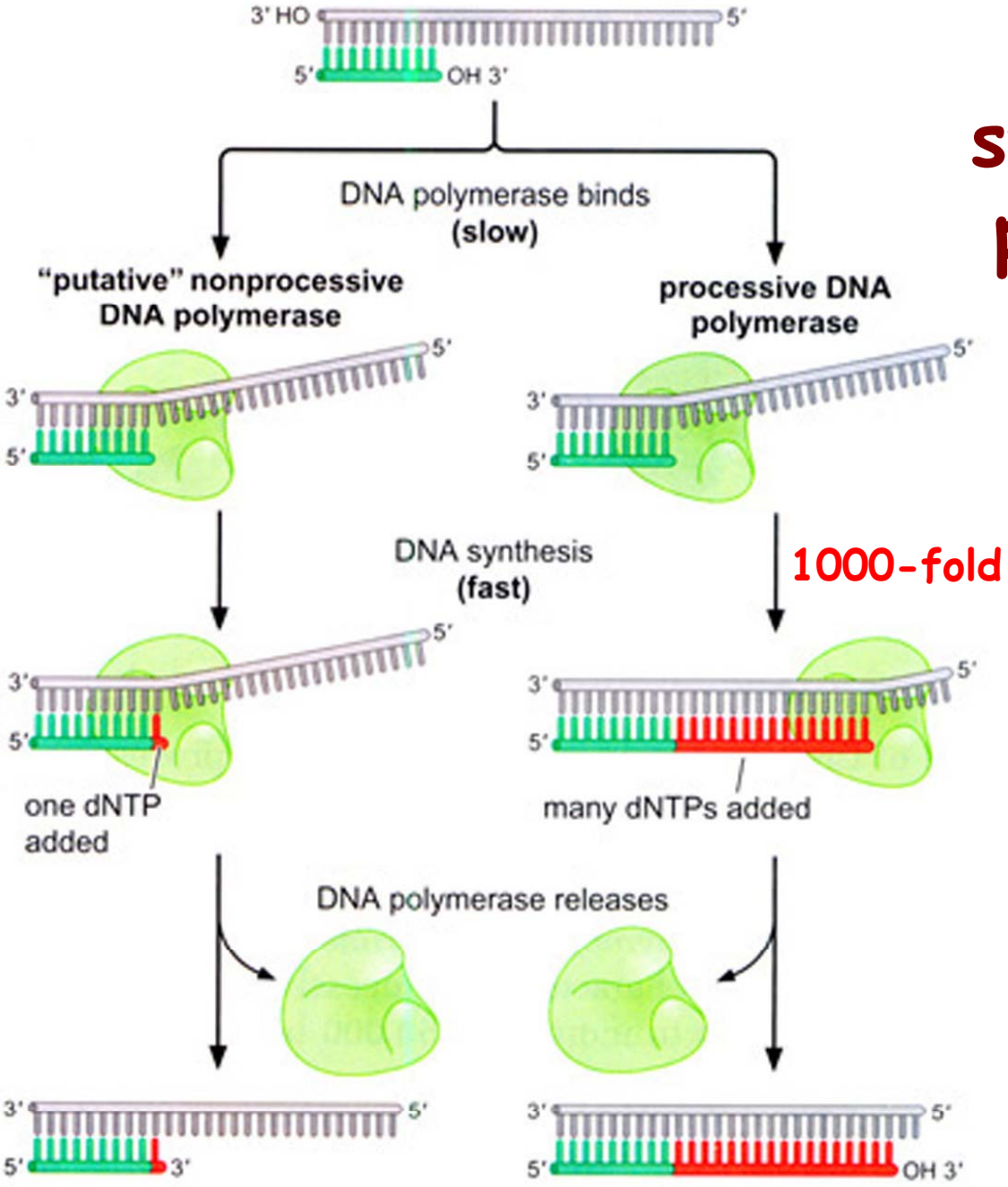
Thumb domain:

1. Not directly involved in catalysis
2. Interacts with the synthesized DNA to maintain correct position of the primer and the active site, and to maintain a strong association between DNA Pol and its substrate.

DNA polymerase are processive enzymes

- **Processivity (持续合成能力)** Processivity is a characteristic of enzymes that operate on polymeric substrates.
- For DNA polymerase, the degree of processivity is defined as *the average number of nucleotides added each time the enzyme binds a primer:template junction* (a few ~50,000).

DNA polymerases synthesis DNA in a processive manner



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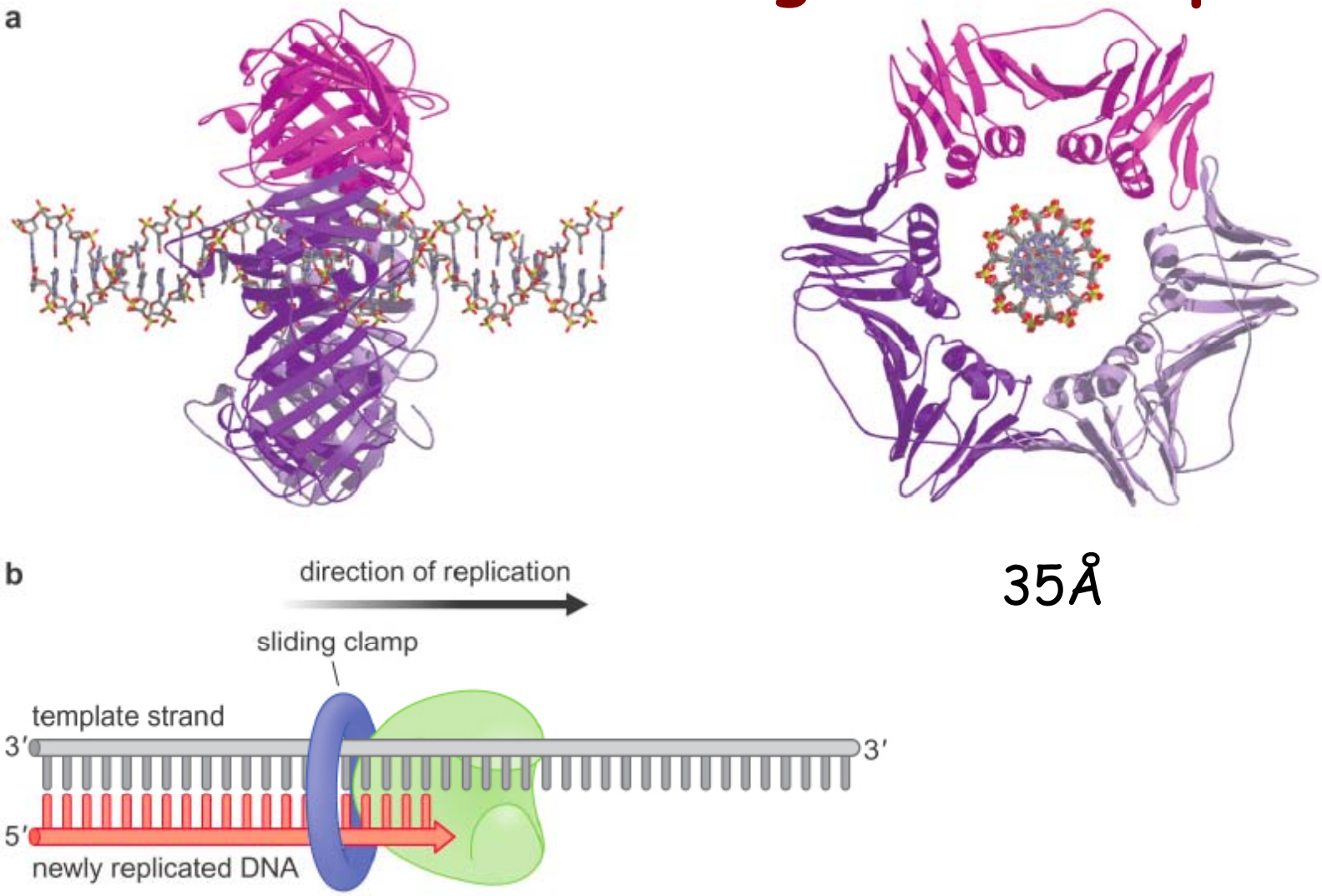
- Increased processivity is facilitated by the ability of DNA polymerase to slide along the DNA template.

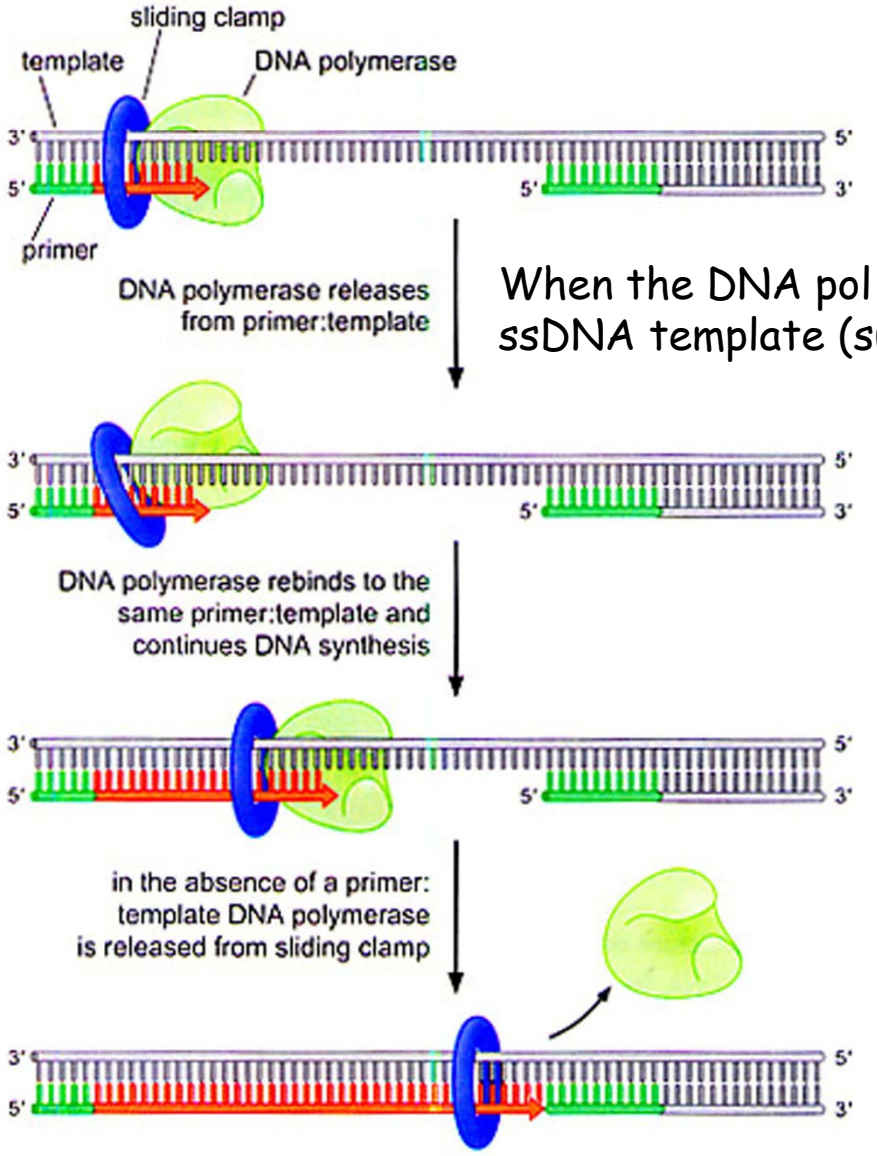
- Further increases in processivity are achieved through interactions between the DNA polymerase and a "sliding clamp" protein that completely encircles the DNA.

Structure of a sliding DNA clamp



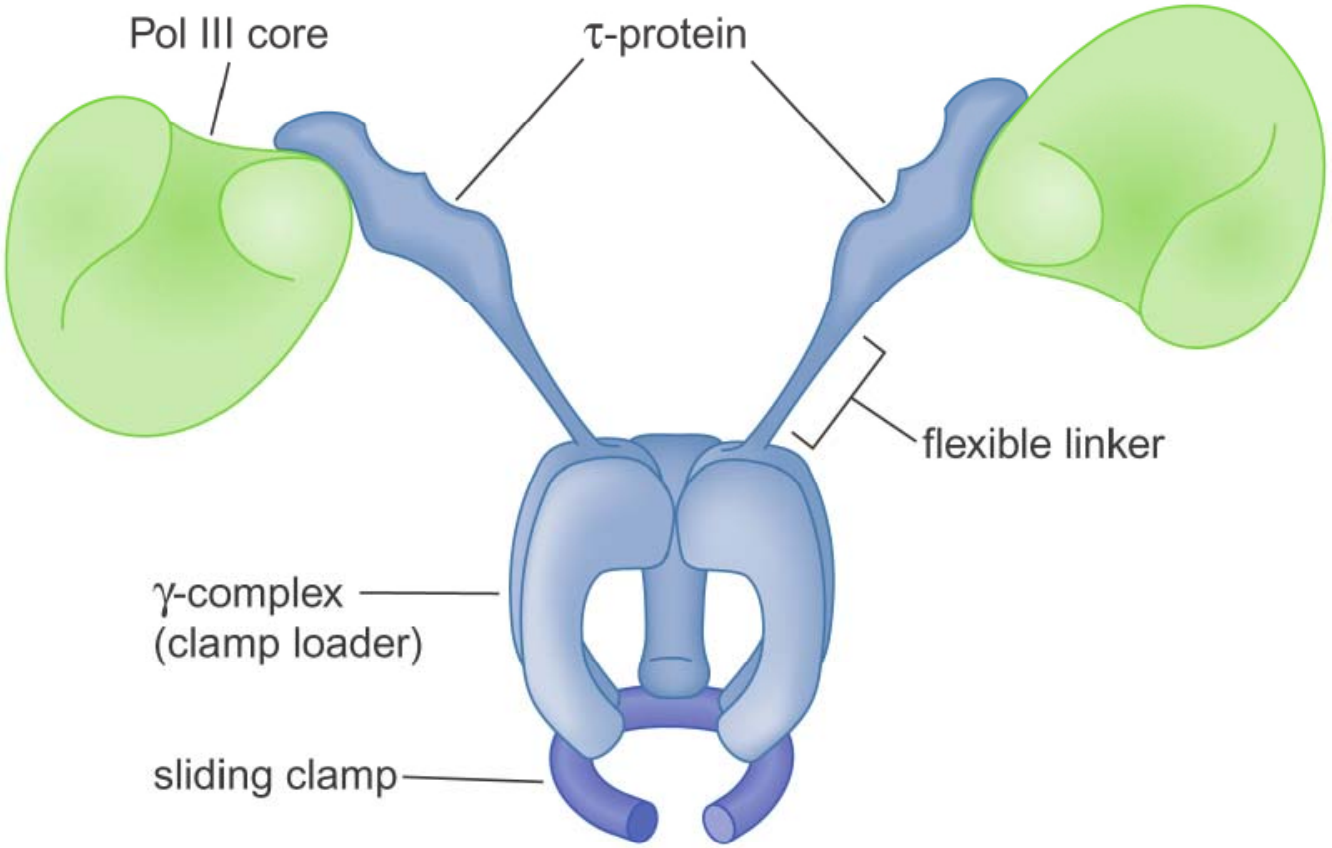
Sliding DNA clamps encircle the newly replicated DNA produced by an associated DNA polymerase.

Sliding clamps dramatically increase DNA polymerase processivity (持续合成能力)



When the DNA pol reaches the end of an ssDNA template (such as Okazaki fragment)

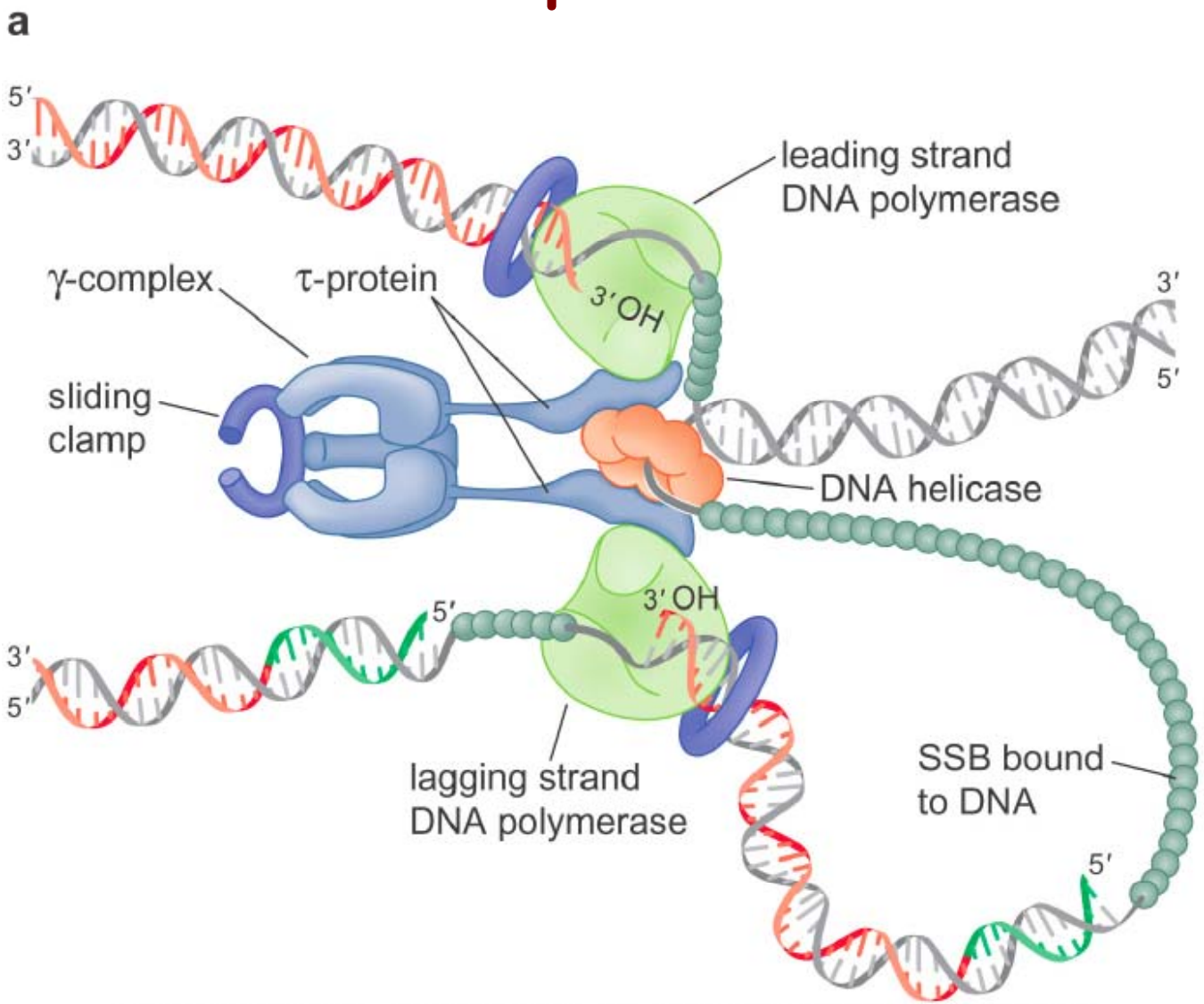
The composition of the DNA Pol III holoenzyme



Three enzymes:

- Two copies of the DNA Pol III core enzyme
- One copy of the γ-complex

The "trombone" model for coordinating replication by two DNA polymerase at the E. coli replication fork



复制叉是不对称的

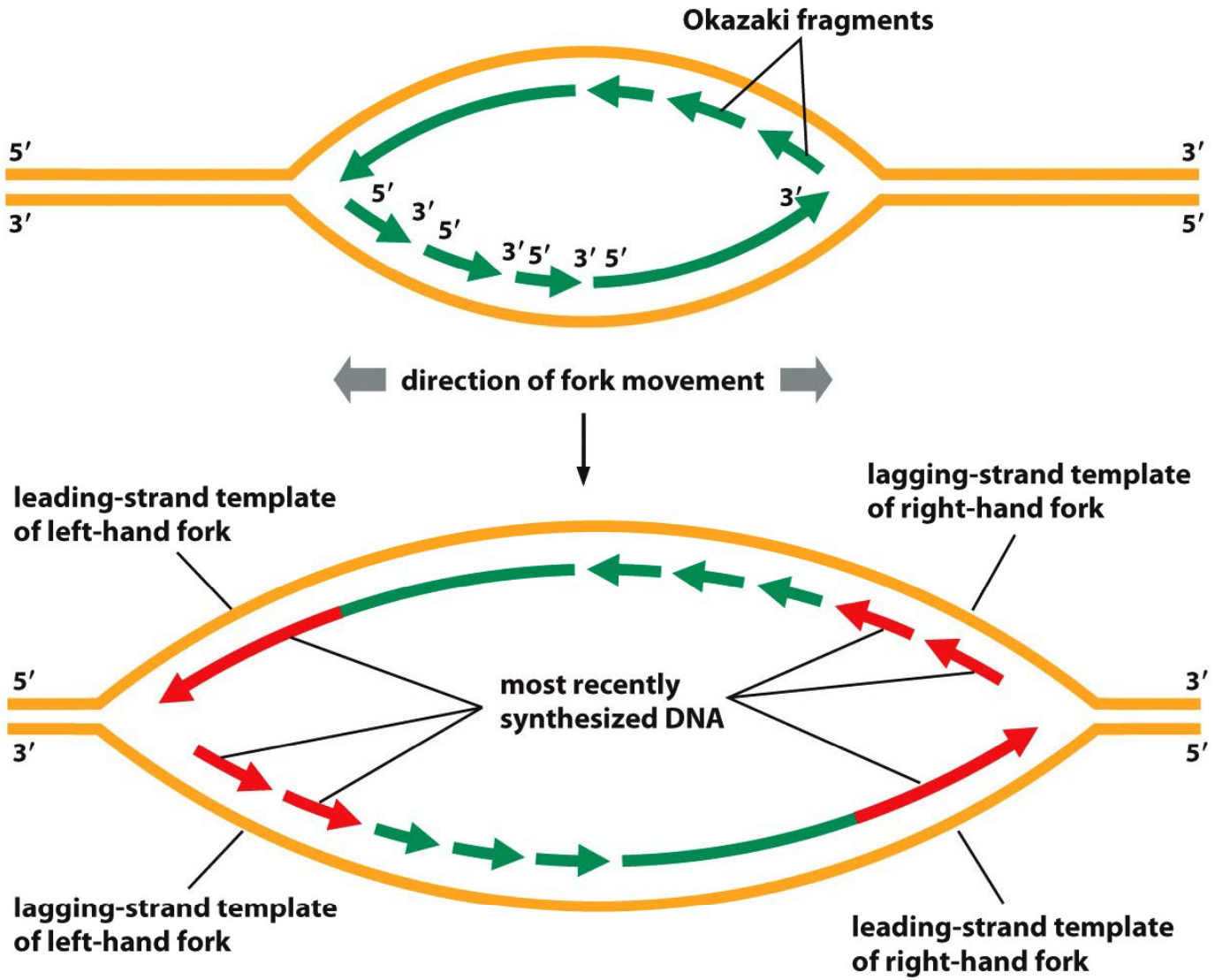
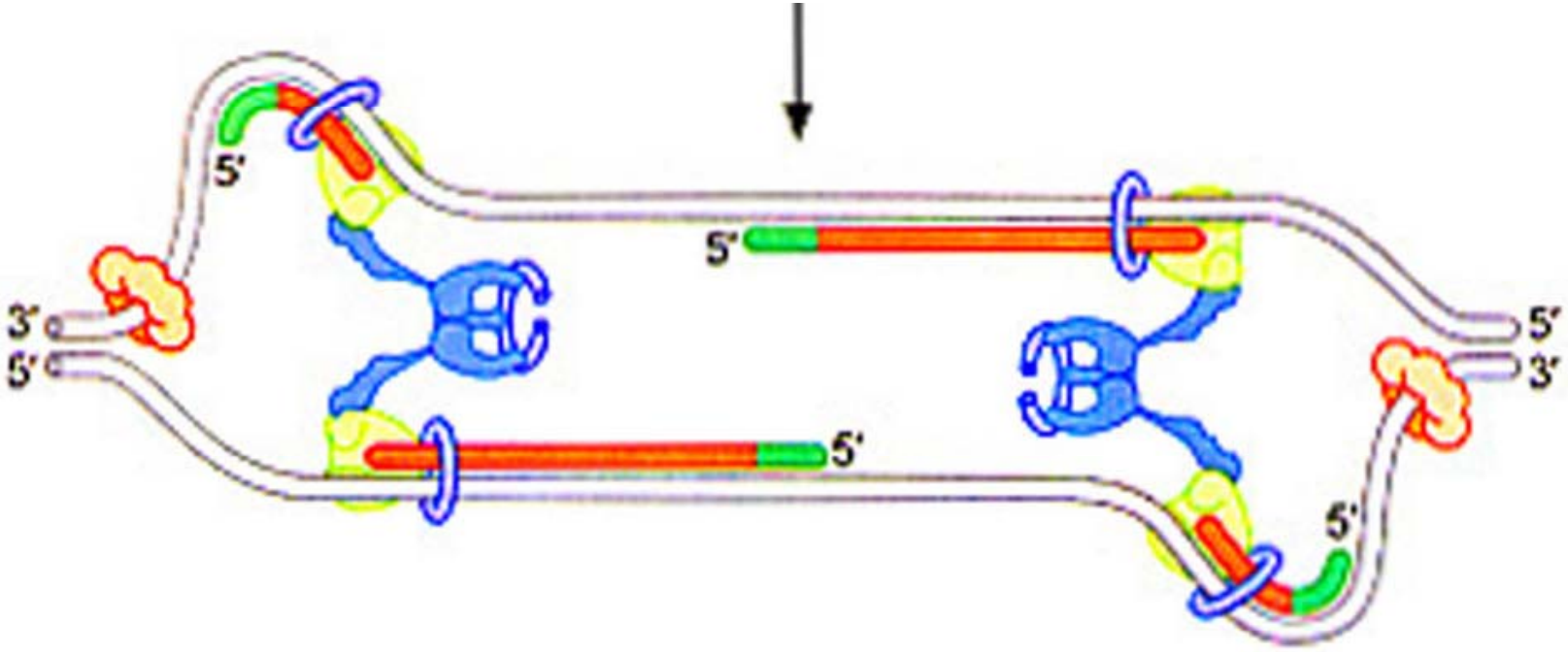
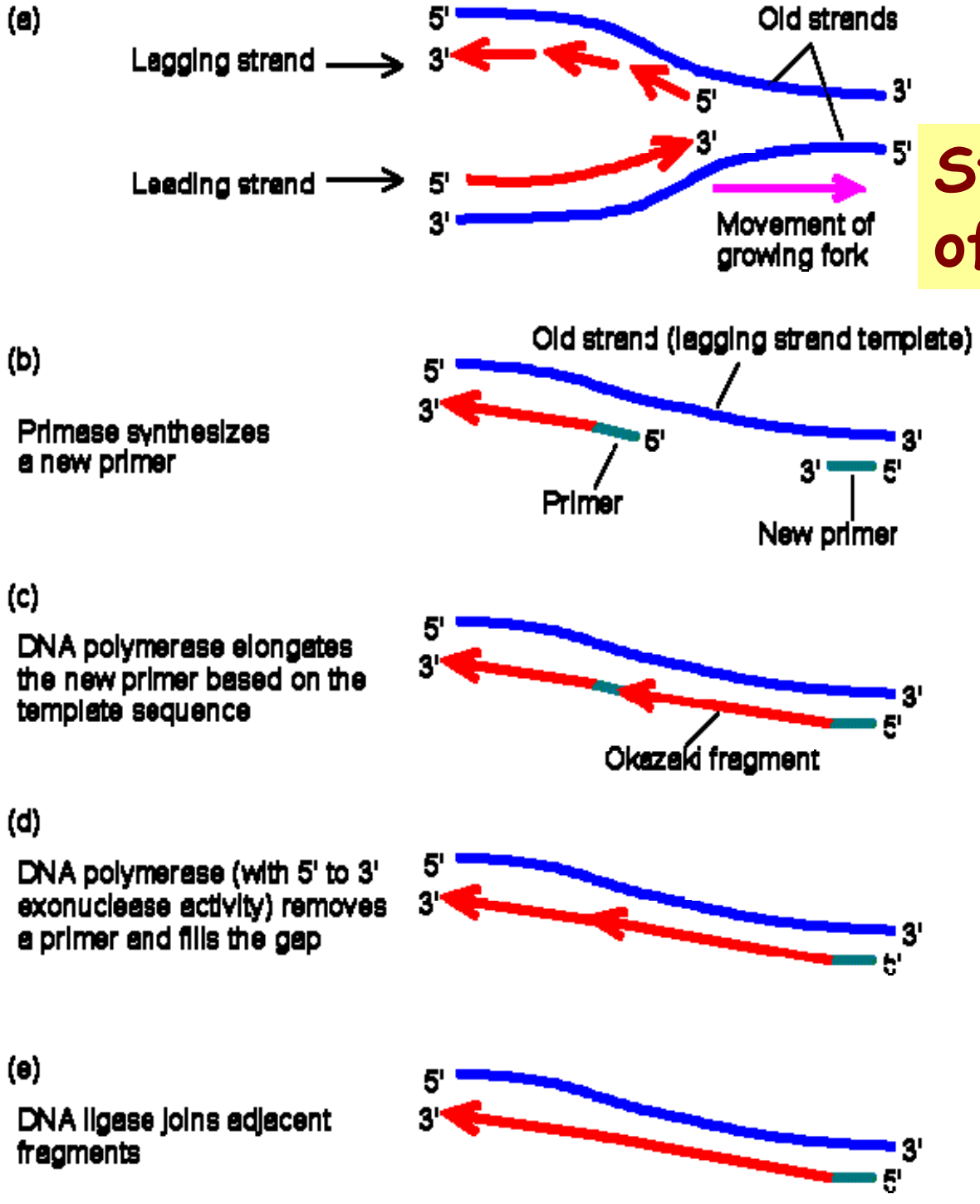


Figure 6-12 Essential Cell Biology 3/e (© Garland Science 2010)

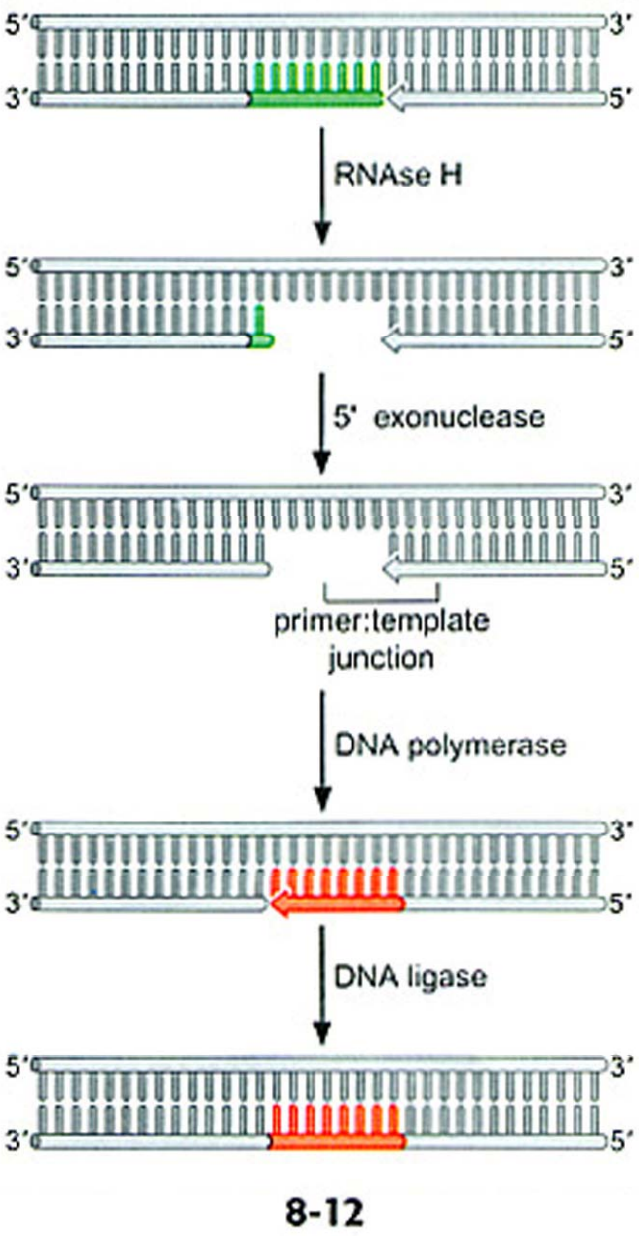
The two DNA helicases function independently



Steps in the synthesis of the lagging strand



Removal of RNA primers from newly synthesized DNA



8-12

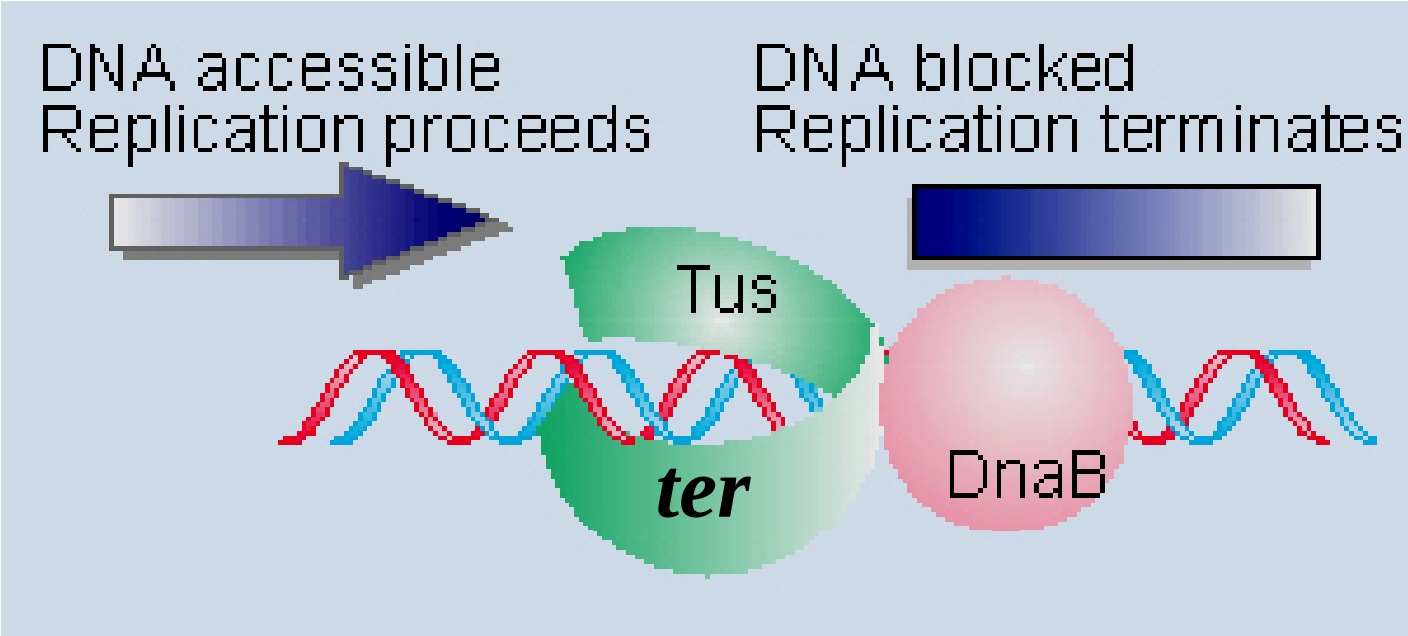
RNase H removes all of the RNA primer except the ribonucleotide directly linked to the DNA end.

An exonuclease removes the final ribonucleotide.

DNA polymerase fills the gap, leaving a a break in the backbone between the 3′OH and 5′ phosphate of the repaired strand.

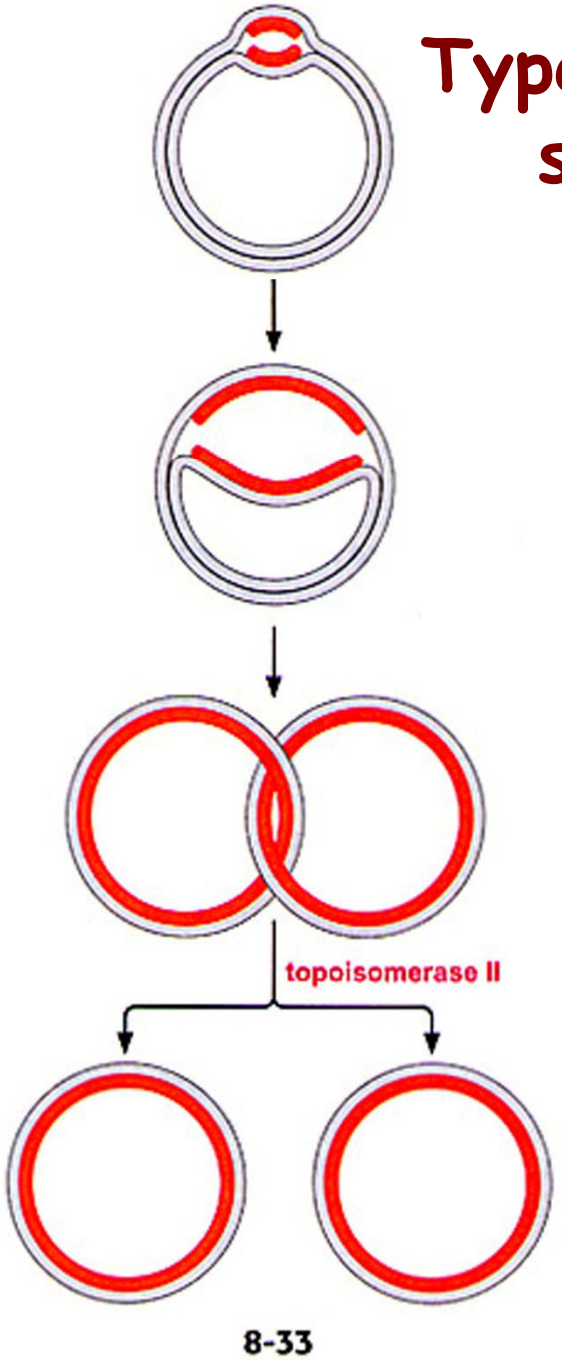
DNA ligase repairs this "nick".

复制的终止



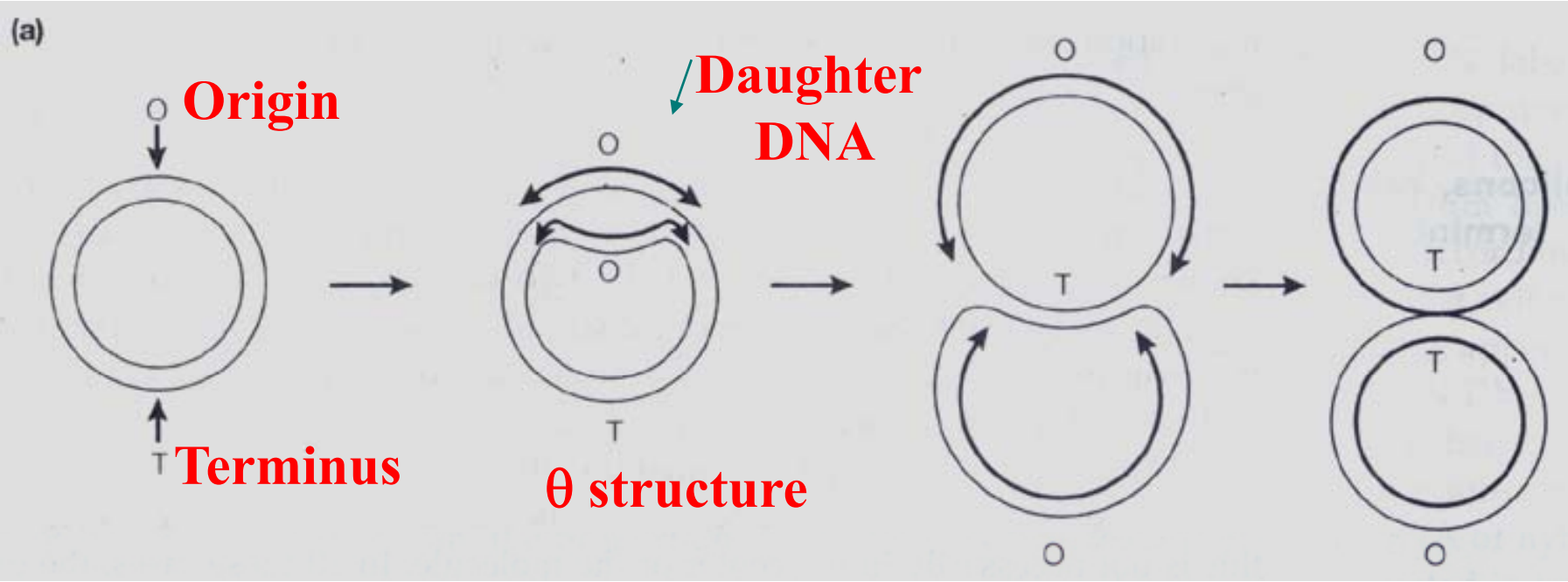
当复制叉前移，遇到**20bp**重复性终止子序列(**Ter**)时，**Ter-Tus**复合物能阻挡复制叉的继续前移，等到相反方向的复制叉到达后在**DNA**拓扑异构酶**IV**的作用下使复制叉解体，释放子链**DNA**。

Type II Topoisomerases are required to separate daughter DNA molecules



For circular chromosome, type II Topoisomerases catalyze a break in one of the two daughter molecules and allow the second daughter molecule to pass through the break.

原核生物DNA的复制特点



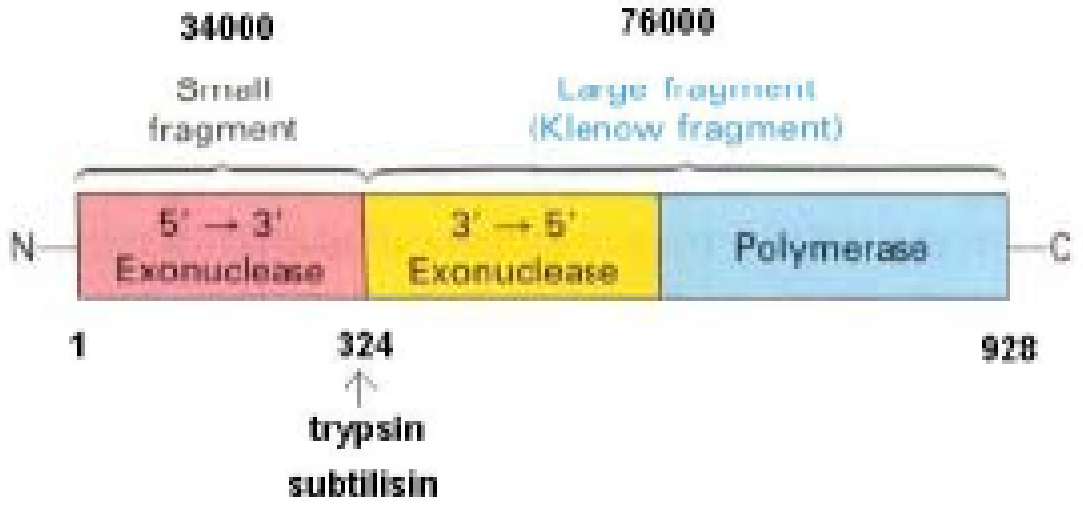
细菌染色体的复制是作为一个单位从唯一的复制起点开始，双向进行的。

DNA聚合酶

大肠杆菌中主要有DNA聚合酶I、II、III、IV和V。

Arthur Kornberg
1959 - Nobel prize in Physiology or Medicine

DNA Polymerase I



DNA聚合酶I (coded by *polA*)不是复制大肠杆菌染色体的主要聚合酶，它有 3’ →5’核酸外切酶活性,保证了DNA复制的准确性。

它的5’ →3’核酸外切酶活性也可用来除去冈崎片段5'端RNA引物，使冈崎片段间缺口消失，保证连接酶将片段连接起来。

DNA Polymerase II

DNA聚合酶II (coded by *polB*)的活性很低，只有DNA聚合酶I的5%，所以也不是复制中主要的酶。

生理功能主要是起修复DNA的作用。

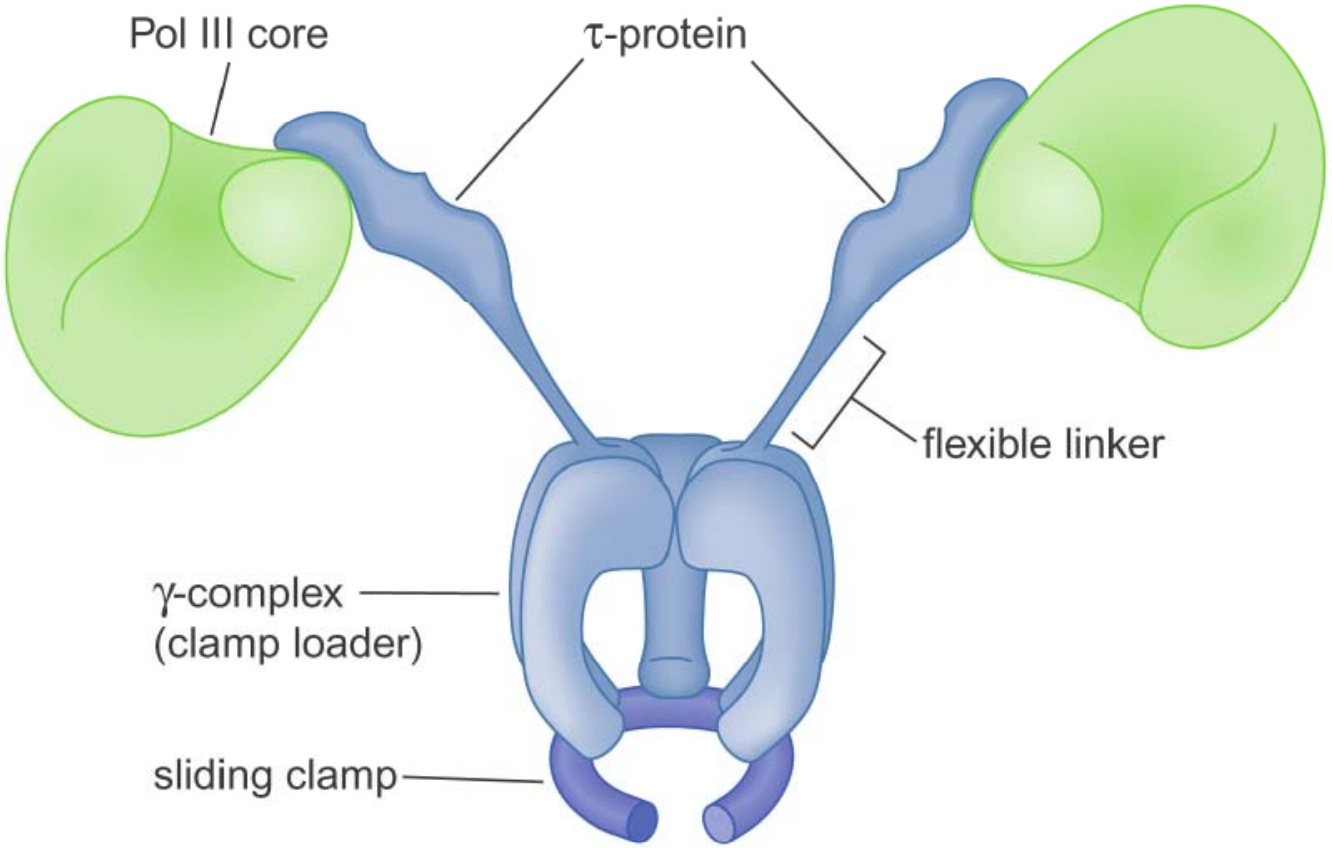
DNA Polymerase III

- **DNA聚合酶III** (coded by *polC*)包含有7种不同的亚单位和9个亚基，其生物活性形式为二聚体。Core enzyme & holoenzyme

- 它的聚合活性较强，为DNA聚合酶I的15倍， 聚合酶II的300倍。

- 它能在引物的3’—OH上以每分钟约5万个核苷酸的速率延长新生的DNA链，是大肠杆菌DNA复制中链延长反应的主导聚合酶。

DNA Polymerase III



- Two copies of the DNA Pol III core enzyme
- One copy of the γ-complex

DNA Polymerase IV& V

- **DNA聚合酶IV和V分别由*dinB*和*umuD'2C*基因编码，**
- **主要在SOS修复过程中发挥功能。**

DNA聚合酶的共同点

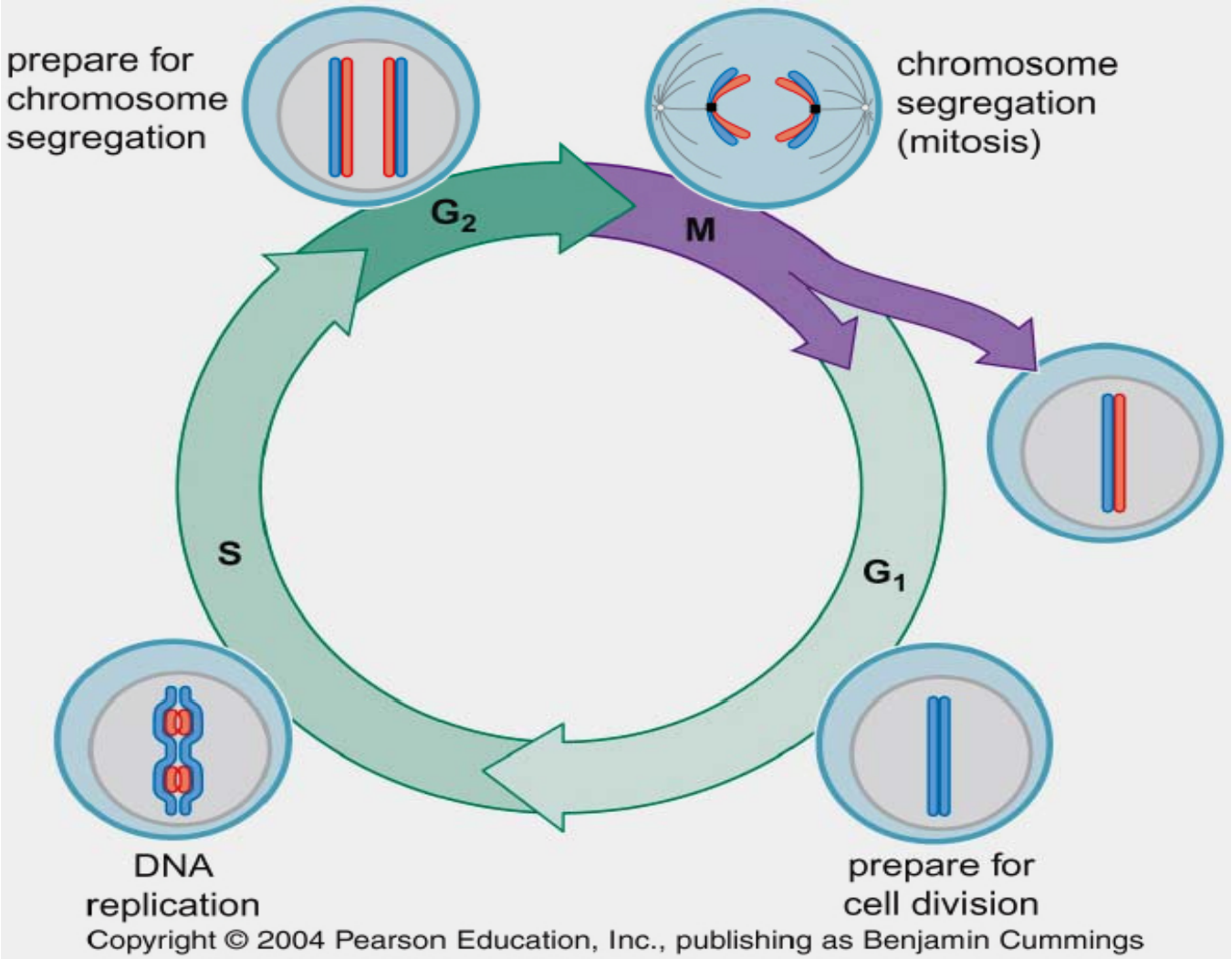
1、都以dNTP为底物。

2、都需要Mg^{2+}激活。

3、聚合时必须有模板链和具有3’-OH末端的引物链。

4、链的延伸都方向为5'→3'。

真核生物DNA的复制


prepare for chromosome segregation
chromosome segregation (mitosis)
G2
M
S
G1
DNA replication
prepare for cell division
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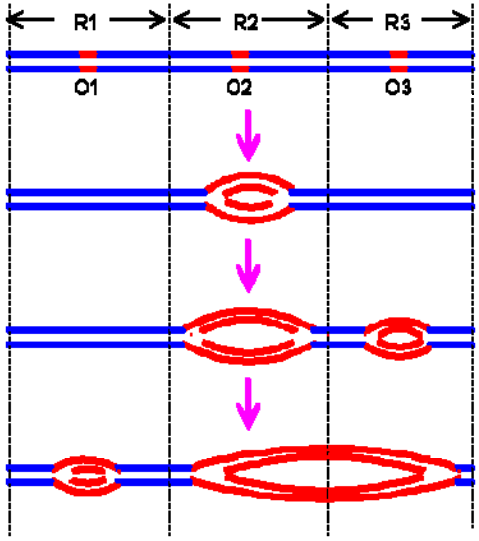
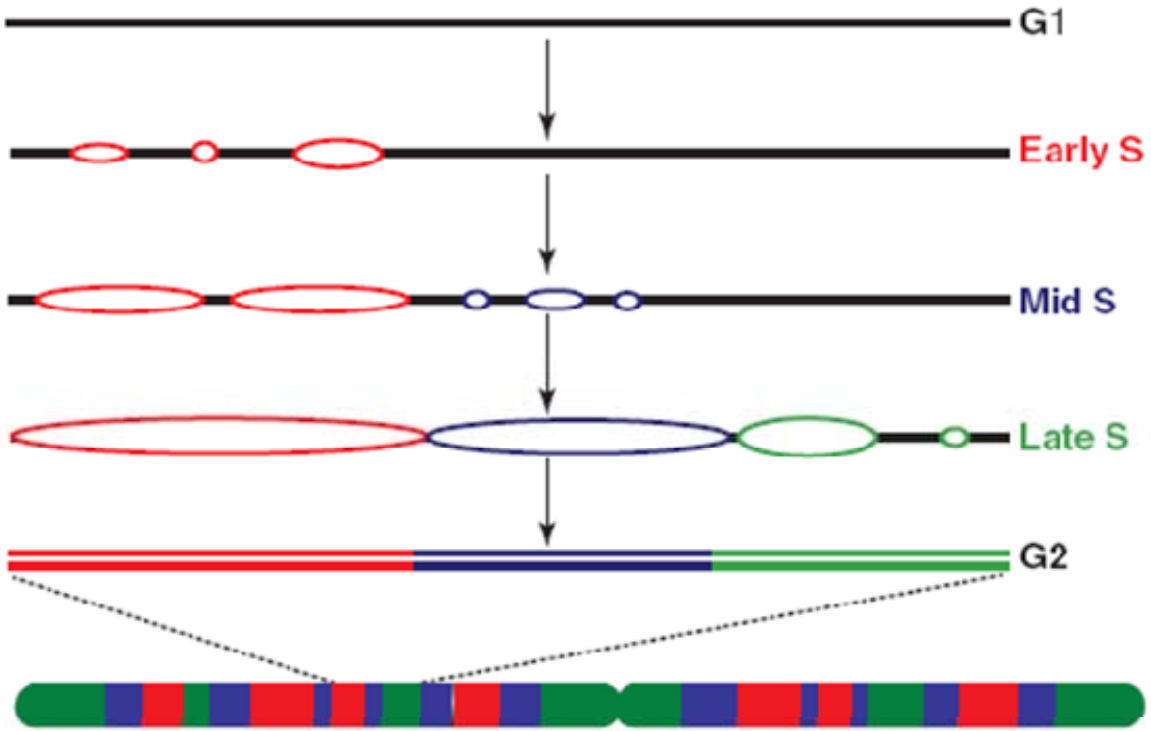
真核生物DNA的复制特点



- 有多处复制起始点；
- 在全部完成复制之前，各个起始点上DNA的复制不能再开始；
- DNA复制只在S期进行。
- 复制子相对较小，为40-100千碱基对。

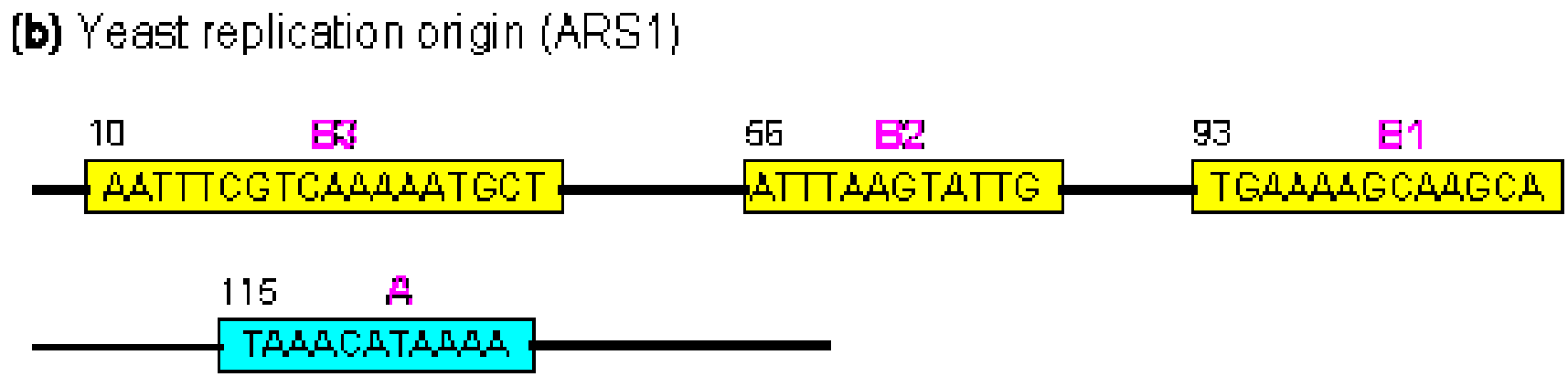
Model for the progression of genome replication—domino model



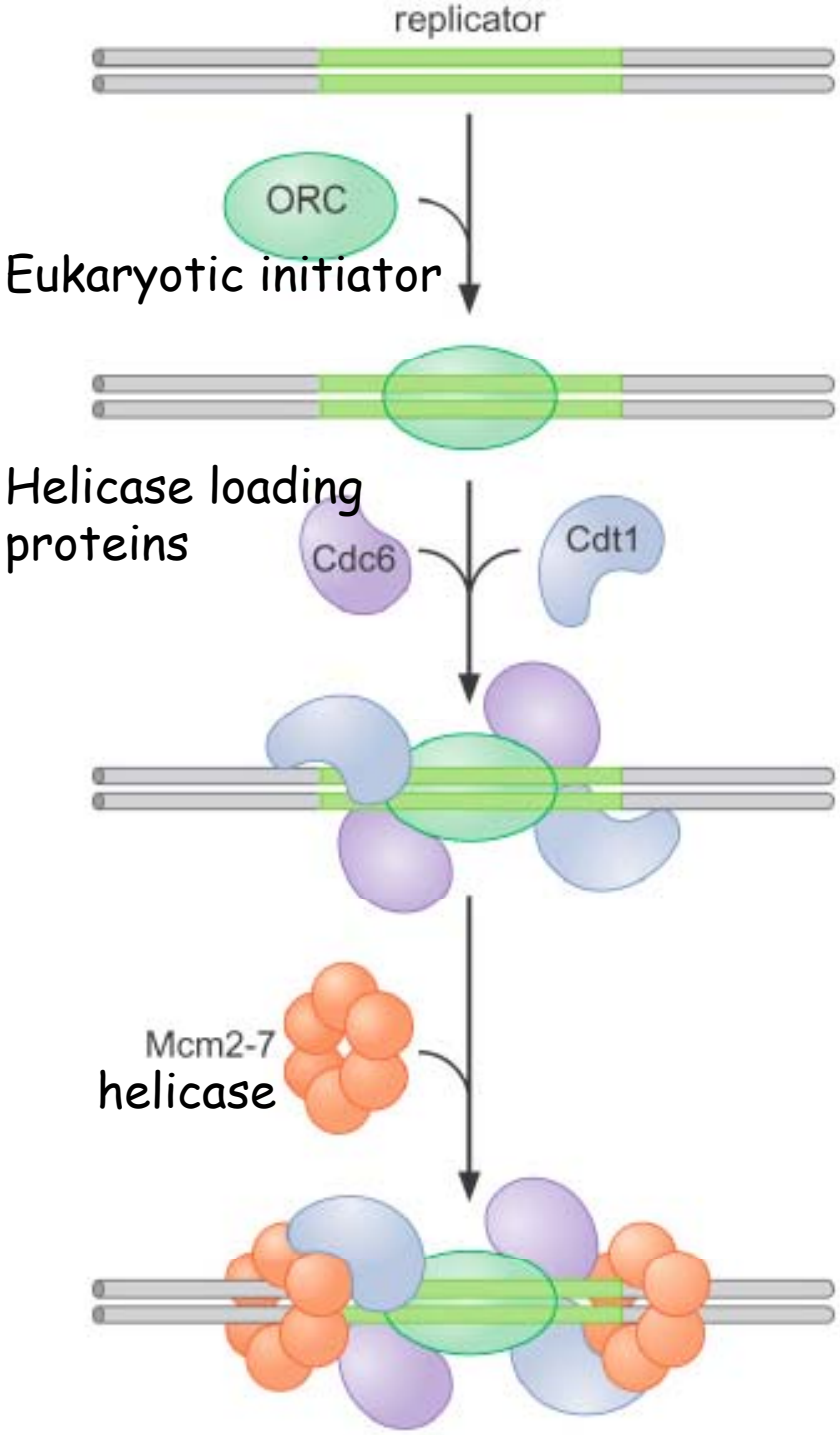
One replicon cluster (red) initiates replication at early S phase and DNA synthesis proceeds bidirectionally (replication bubbles). This activates initiation of neighboring replicon clusters (blue), which in turn activate replication at later replicon clusters (green) until the whole chromosome (see scheme below) is fully duplicated in G2 phase.

Cold Spring Harb Perspect Biol 2010;2:a000737

真核生物DNA的复制子被称为ARS (autonomously replicating sequences)，长约150bp左右，包括数个复制起始必需的保守区。

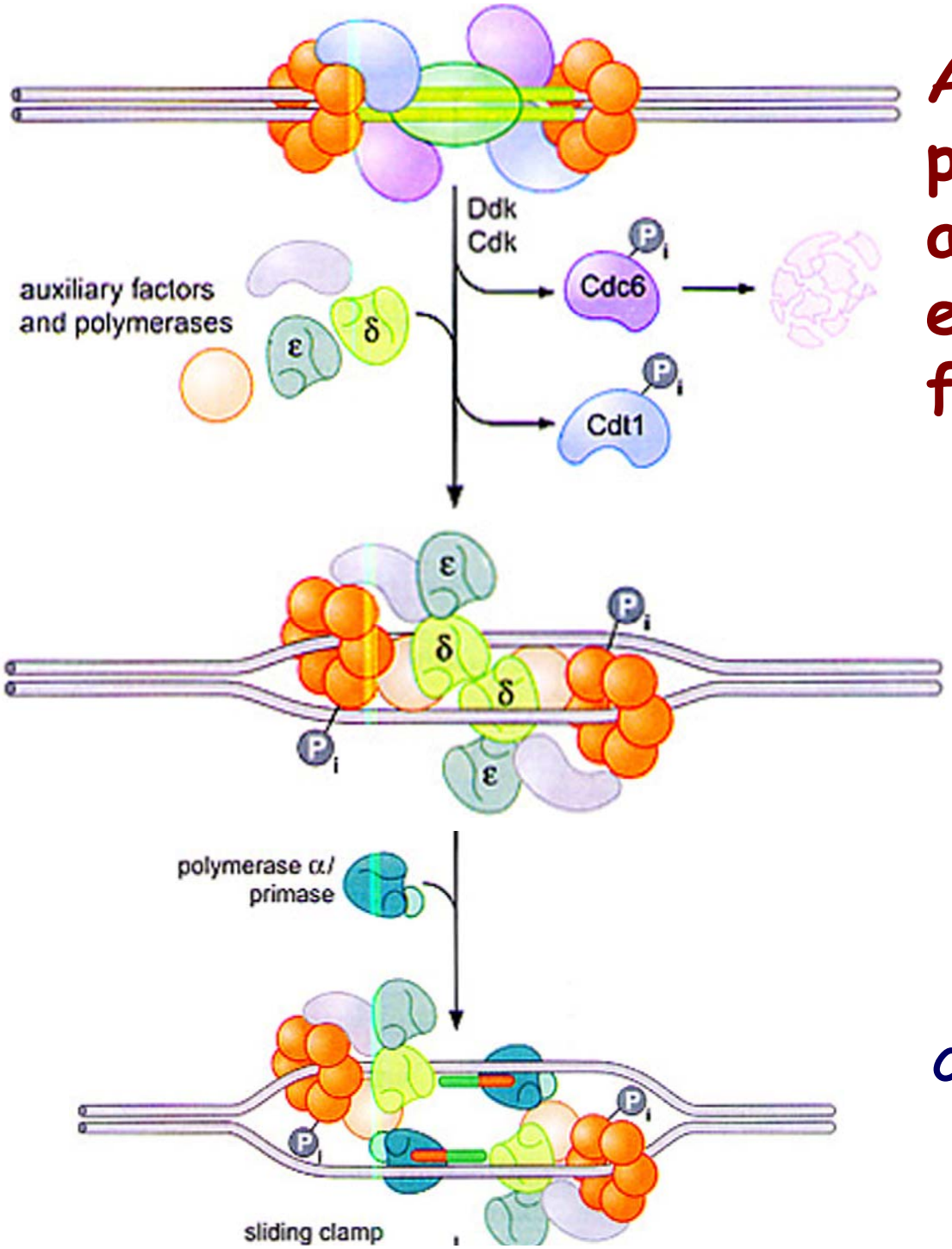


真核生物DNA复制的起始需要起始点识别复合物（origin recognition complex，ORC）参与，ORC结合于ARS，它是由6种蛋白质组成的启动复合物。



Pre-replicative complex formation and activation is regulated to allow only a single round of replication during each cell cycle

- The assembly of the pre-RC in G1 is an ordered process that is initiated by the association of the ORC with the replicator.
- Once bound to the replicator, ORC recruits at least two additional proteins, Cdc6 and Cdt1.
- These three proteins function together to recruit the DNA helicase(Mcm2-7 complex) to complete the formation of the pre-RC.

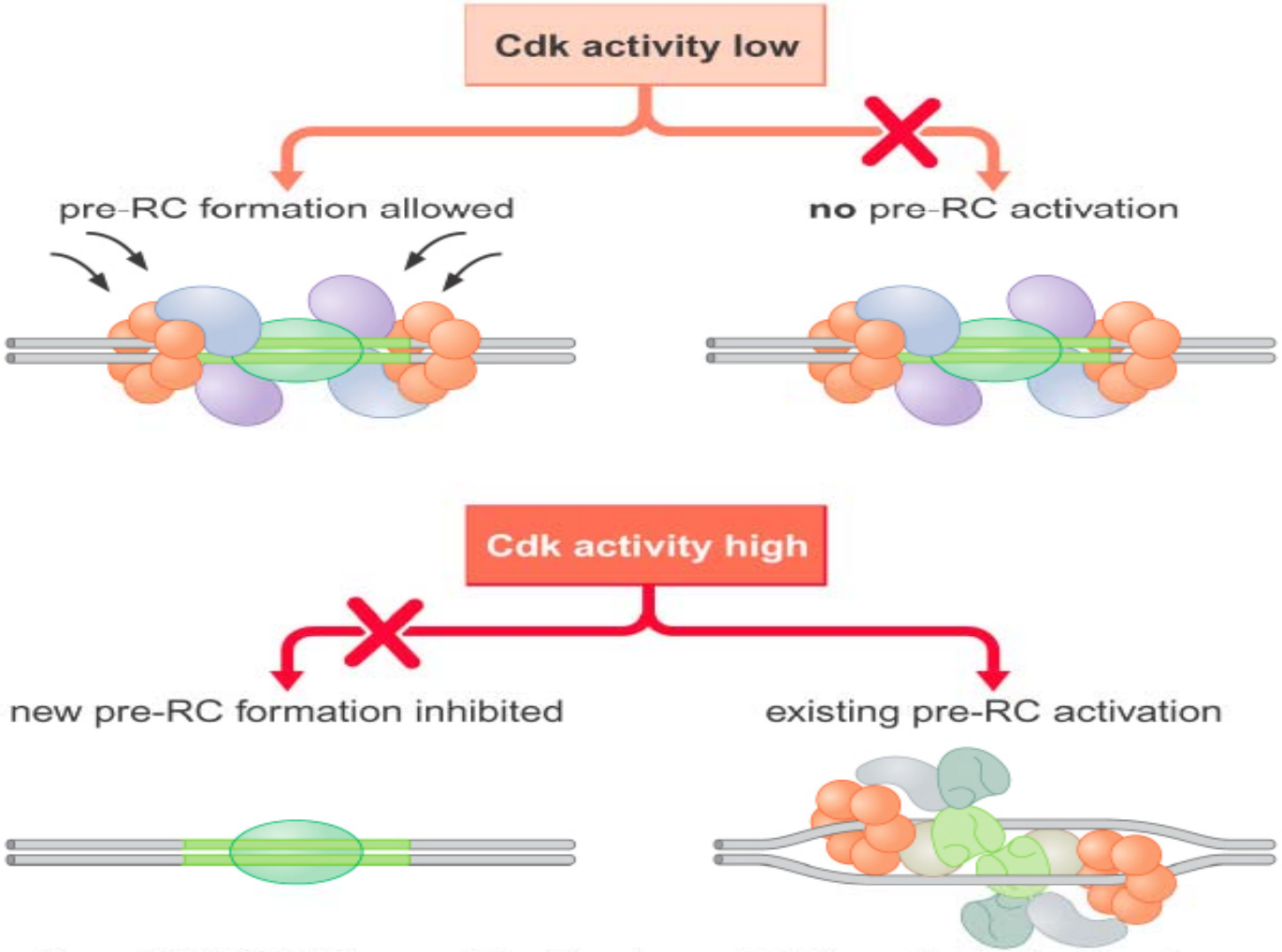


Activation of the pre-RC leads to the assembly of the eukaryotic replication fork

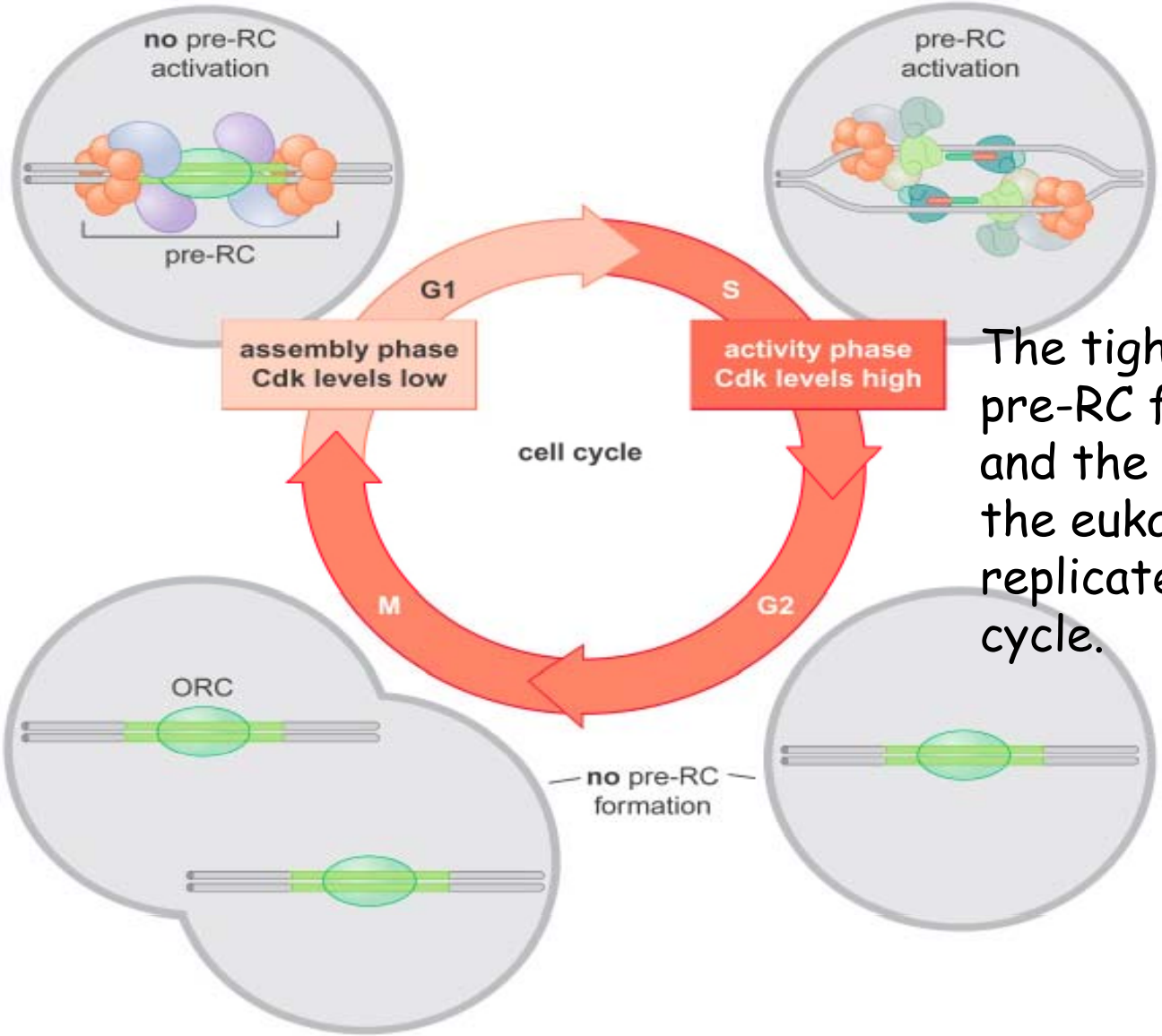
Pre-RCs are activated to initiate replication by two protein kinases (Cdk and DdK), which are only activated when cells enter S phase.

Cdk: cyclin-dependent kinase

High Cdk activity is required for existing pre-RC to initiate DNA replication



Cell cycle regulation of Cdk activity and pre-RC formation



The tight connection between pre-RC function, Cdk levels, and the cell cycle ensures that the eukaryotic genome is replicated only once per cell cycle.

真核生物**DNA**复制叉的移动速度大约只有**50bp/**秒。

因此，人类**DNA**中每隔**30,000-300,000bp**就有一个复制起始位点。

- 已发现的真核生物**DNA**聚合酶有**15**种以上。

- 在哺乳动物细胞中主要有**5种DNA聚合酶**，分别称为**DNA**聚合酶α、β、γ、δ和ε 。

真核生物DNA聚合酶的特性比较

性质	DNA聚合酶α	DNA聚合酶β	DNA聚合酶γ	DNA聚合酶δ	DNA聚合酶ε
亚基数	4	1	2	2-3	≥1
在细胞内分布	核内	核内	线粒体	核内	核内(?)
功能	DNA引物合成	损伤修复	线粒体DNA复制	主要DNA复制酶	DNA复制(?)
3'→5'外切	无	无	有	有	有
5'→3'外切	无	无	无	无	无

DNA聚合酶α主要参与引物合成。

DNA聚合酶β活性水平稳定，主要在DNA损伤的修复中起作用。

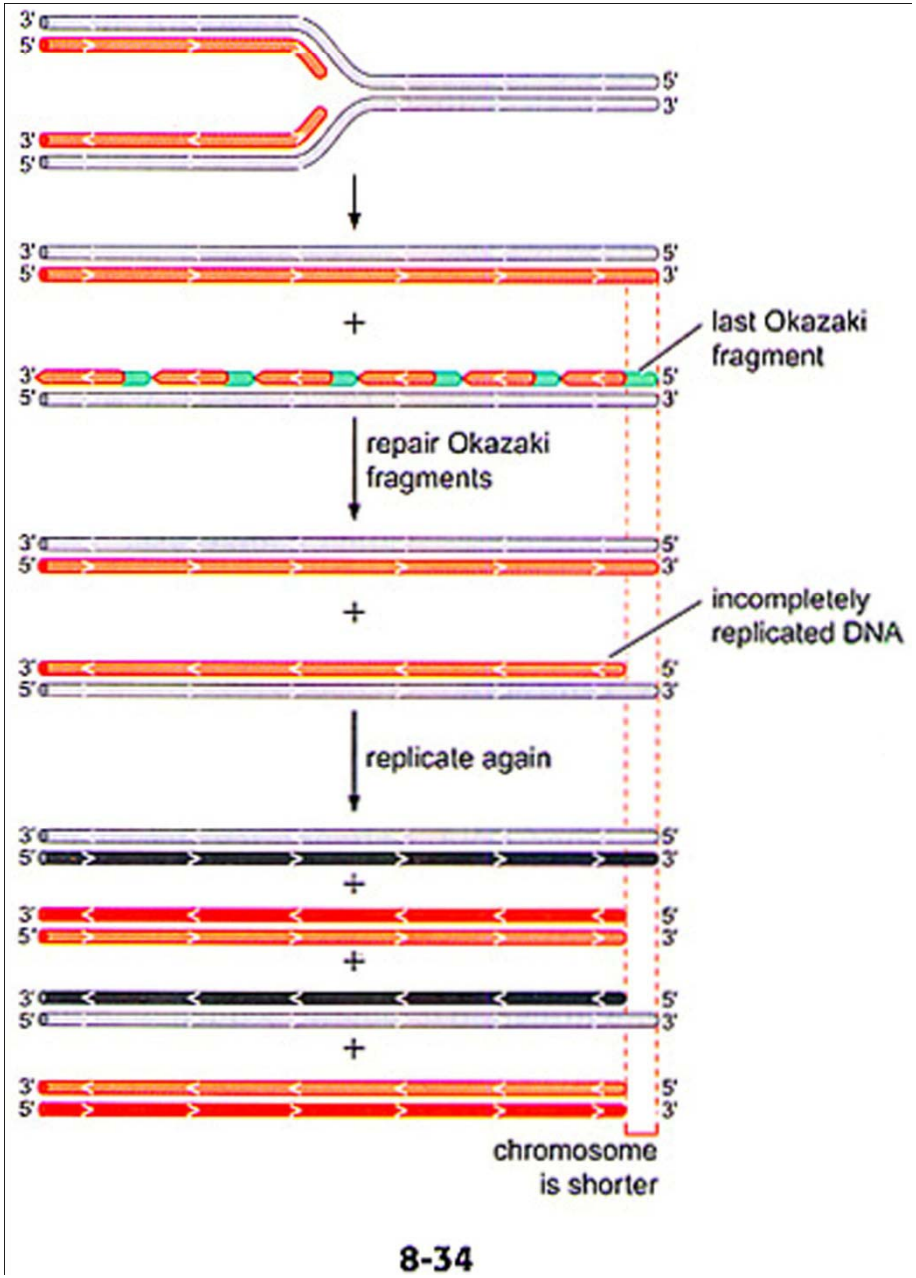
DNA聚合酶δ主要负责DNA的复制。

DNA聚合酶ε与后随链合成有关，在DNA合成过程中核苷切除以及碱基的切除修复中起着重要的作用。

DNA聚合酶γ在线粒体DNA的复制中发挥作用。

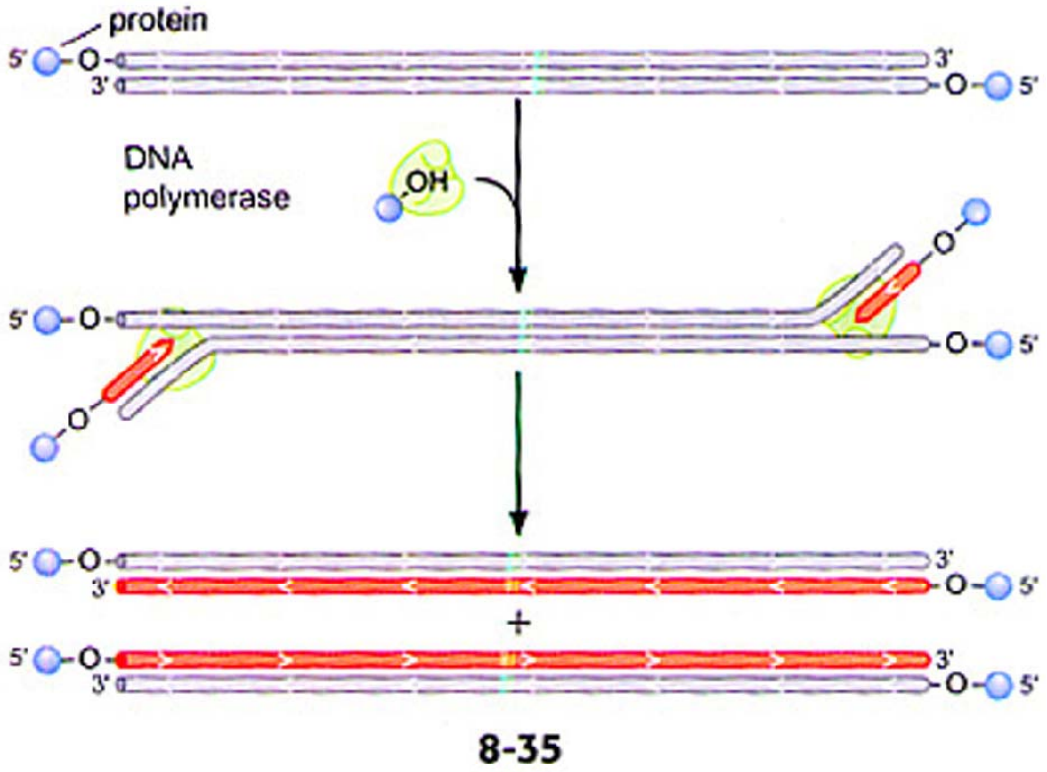
真核生物中还存在ζ、η、ι和κ等几种**DNA**聚合酶，它们承担着修复损伤的功能，但这些修复酶的忠实性都很低。

末端复制问题



后随链复制机器到达染色体末端时，有时引物酶没有足够的空间去合成新的RNA引物，导致复制的不完整和后随链DNA产物上3’端形成一小段单链DNA。

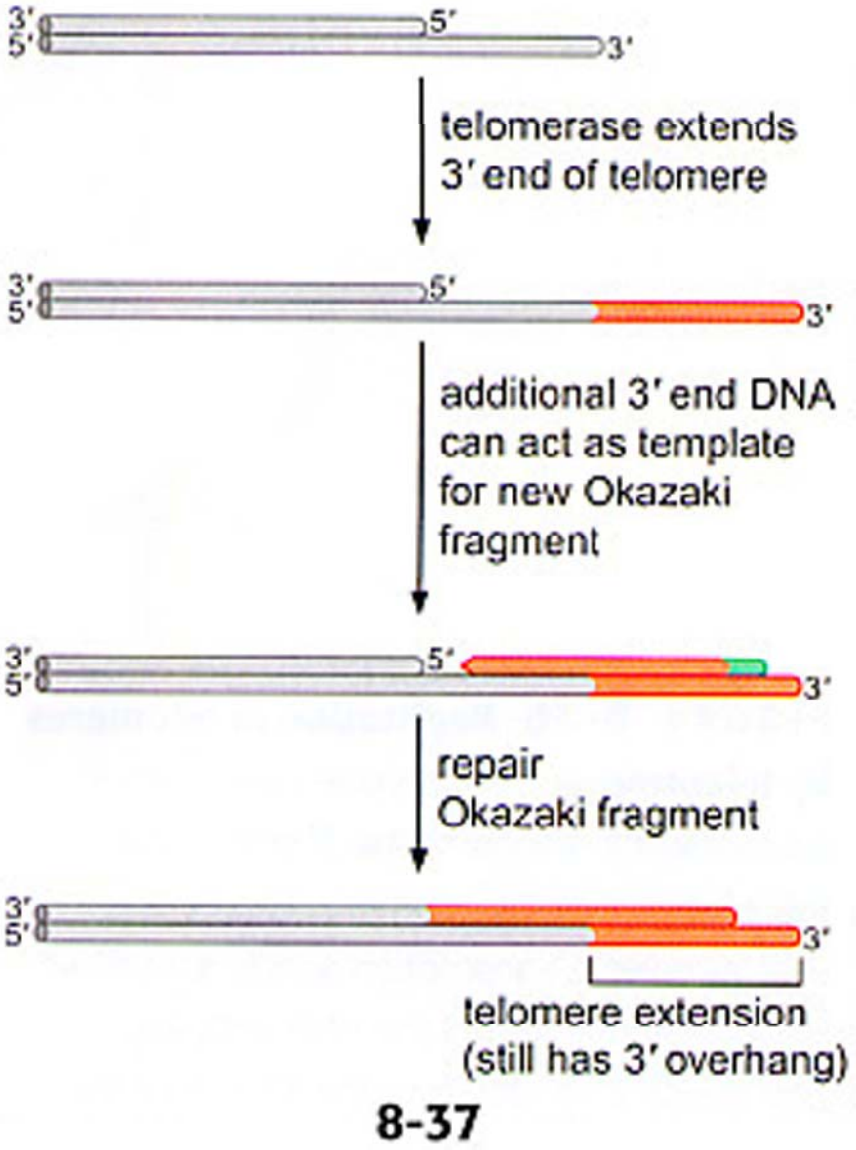
蛋白质引物是解决末端复制问题的一个方法



•通过与DNA聚合酶和模板3’端的结合，蛋白质提供具引物作用的OH基团以起始DNA的合成。

•发生在具有线性染色体的细菌和细菌病毒和动物病毒。

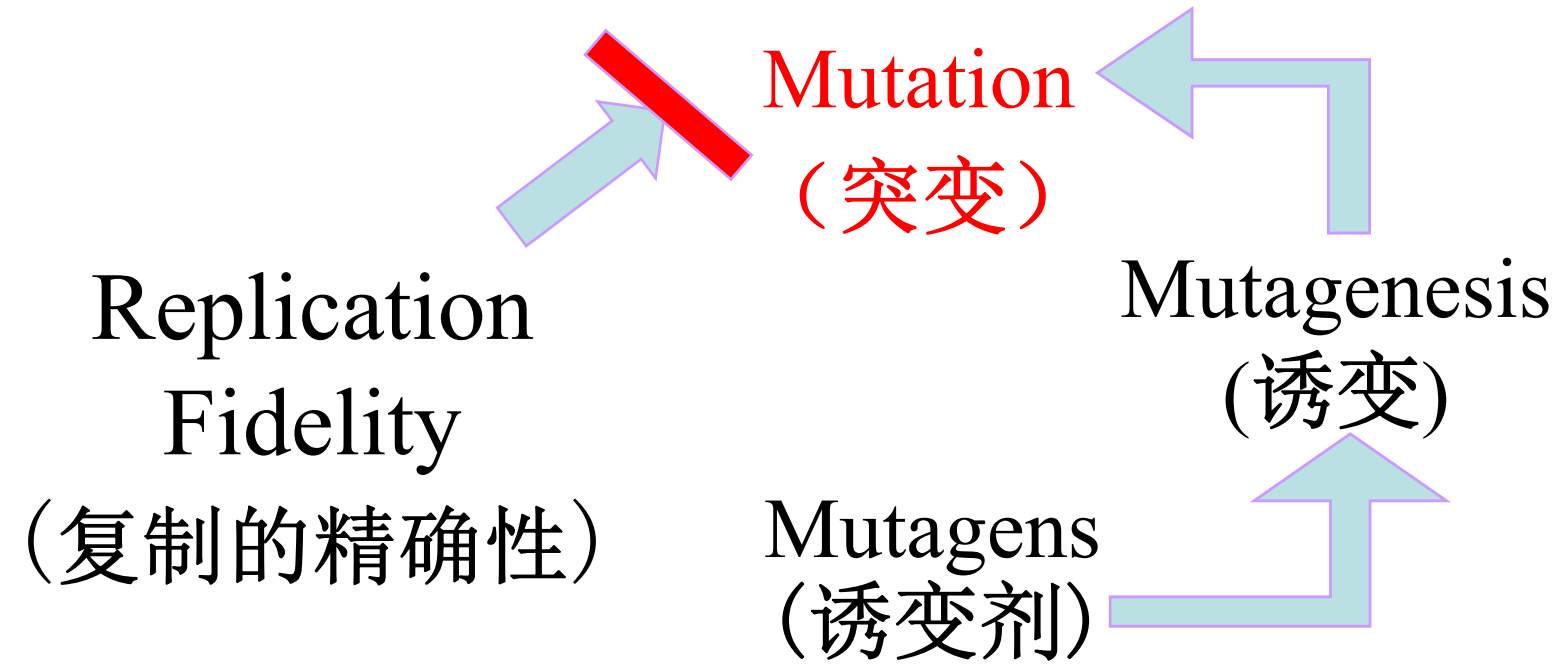
端粒使真核生物染色体末端完成DNA合成



- 端粒酶是一种新型的DNA聚合酶，它不需要外源模板。
- 端粒酶既含有蛋白质又含有RNA。
- 它利用其RNA成分为模板，将端粒序列添加到染色体末端的3’端
- 端粒酶和后随链复制机器的作用，能够保证端粒维持在足够的长度来保护染色体末端不会变的太短。

DNA修复
(DNA Repair)

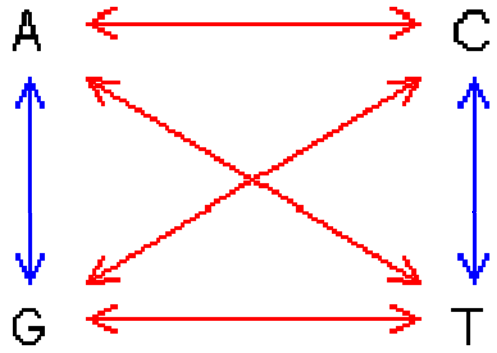
Mutation



Mutation: *Permanent, heritable alterations in the base sequence of DNA.*

The substitution mutation(替换突变)

(a)



Transition(转换): Purine or pyrimidine is replaced by the other.

Transversion(颠换): a purine is replaced by a pyrimidine or vice verse.

(b)

Silent mutation 沉默突变	Missense mutation 错义突变	Nonsense mutation 无义突变
TGT → TGC	TGT → TGG	TGT → TGA
Cys → Cys	Cys → Trp	Cys → Stop

单个核苷酸的改变导致镰状细胞贫血病

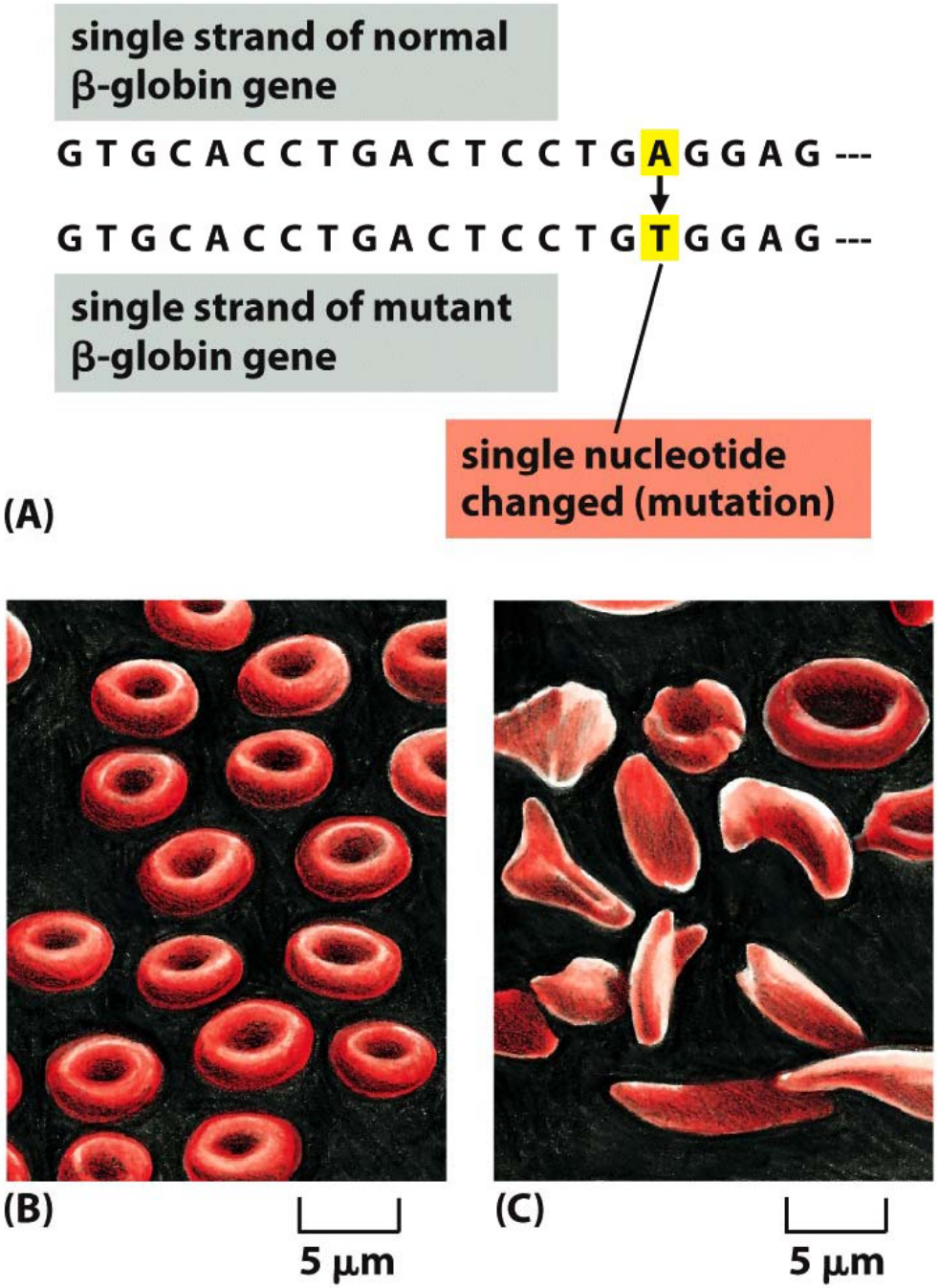


Figure 6-19 Essential Cell Biology 3/e (© Garland Science 2010)

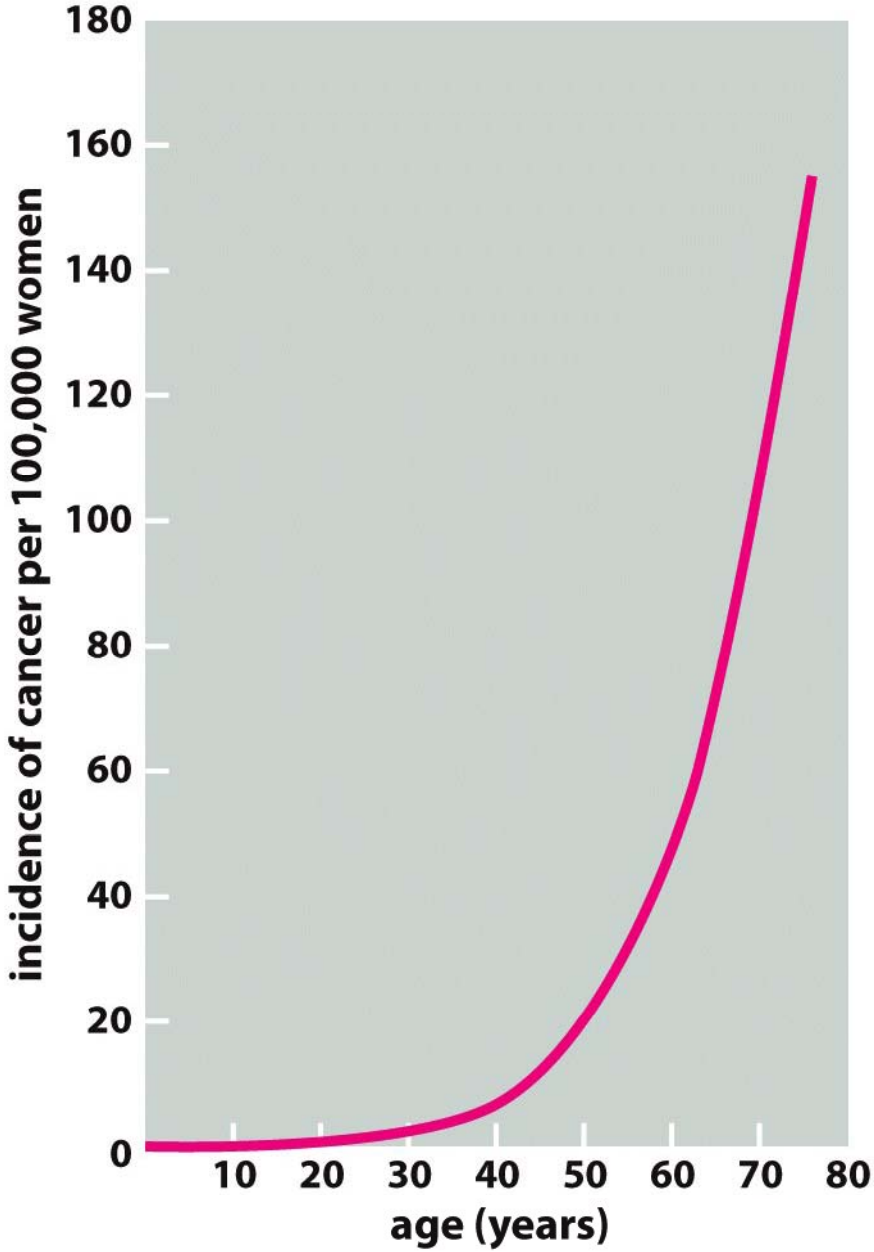


Figure 6-20 Essential Cell Biology 3/e (© Garland Science 2010)

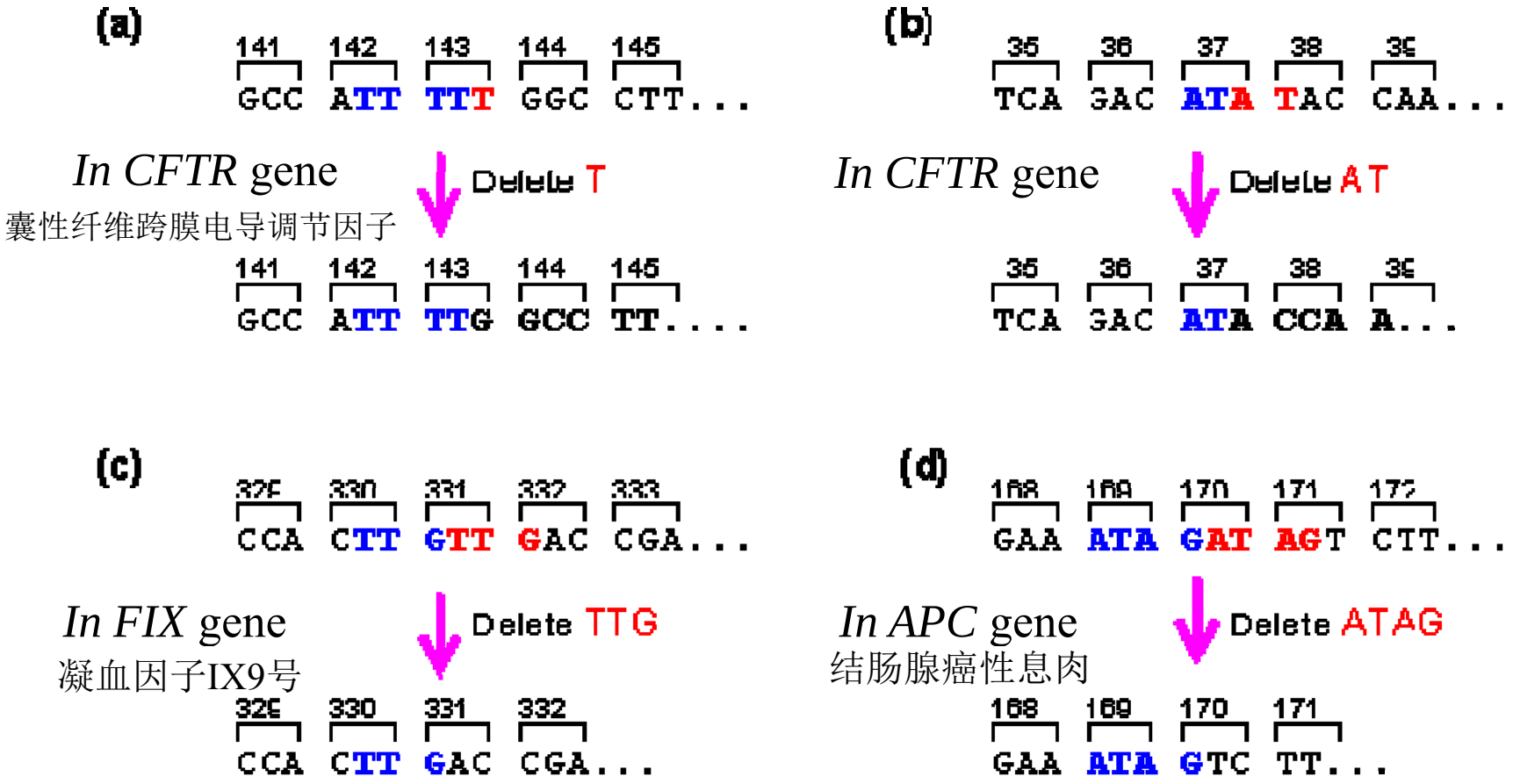
癌症发病率是随着年龄增长的函数而急剧增加

Deletion or Insertion
（缺失或插入）

The elimination or addition of one or more nucleotides in a DNA region.

It may cause **frameshift** (移码突变) producing a **non-functional protein**.

Real examples of deletion mutations which cause diseases



Serious Consequences of Insertion Mutation

Disease	Gene Location	Repeat Sequence	Normal Repeat Number	Mutated Repeat Number
Huntington disease	4p16.3	CAG	9 - 35	37 - 100
Kennedy disease	Xq21	CAG	17 - 24	40 - 55
SCA1	6p23	CAG	19 - 36	43 - 81
DRPLA	12p	CAG	7 - 23	> 49
Fragile X site A	Xq27.3	CGG	6 - 54	> 200
Fragile X site E	Xq28	CCG	6 - 25	> 200
Fragile X site F	Xq28	GCC	6 - 29	> 500
Myotonic dystrophy	19q13	CTG	5 - 35	50 - 4000

Replication fidelity
(复制的精确性)

Important for preserve the genetic information from one generation to the next.

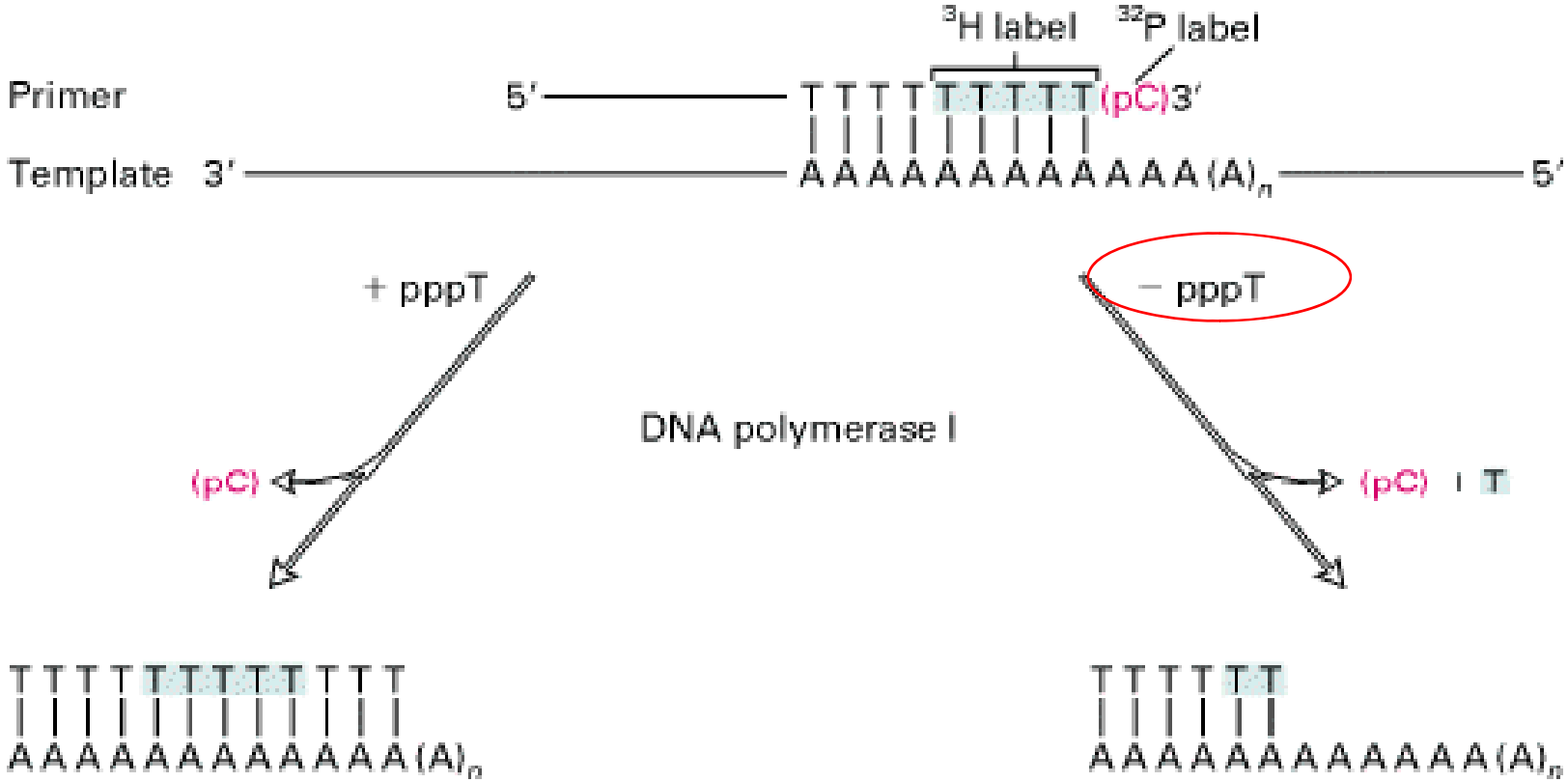
Mutation relevant

Spontaneous errors in DNA replication is very rare, one error per 10^{10} base in *E. coli*, which is much less than the error that DNA polymerase III introduced.

This increased accuracy *in vivo* is largely due to the ***proofreading function*** of *E. coli* DNA polymerases

Proofreading refers to any mechanism for correcting errors in protein or nucleic acid synthesis that involves scrutiny of individual units after they have been added to the chain.

Proofreading function of *E. coli* DNA polymerases



Experimental demonstration of the proofreading function of *E. coli* DNA polymerase I.

[See A. Kornberg and T. A. Baker, 1992, *DNA Replication,* 2d ed., W. H. Freeman and Company.]

DNA聚合酶含有DNA合成和校读两个不同的位点

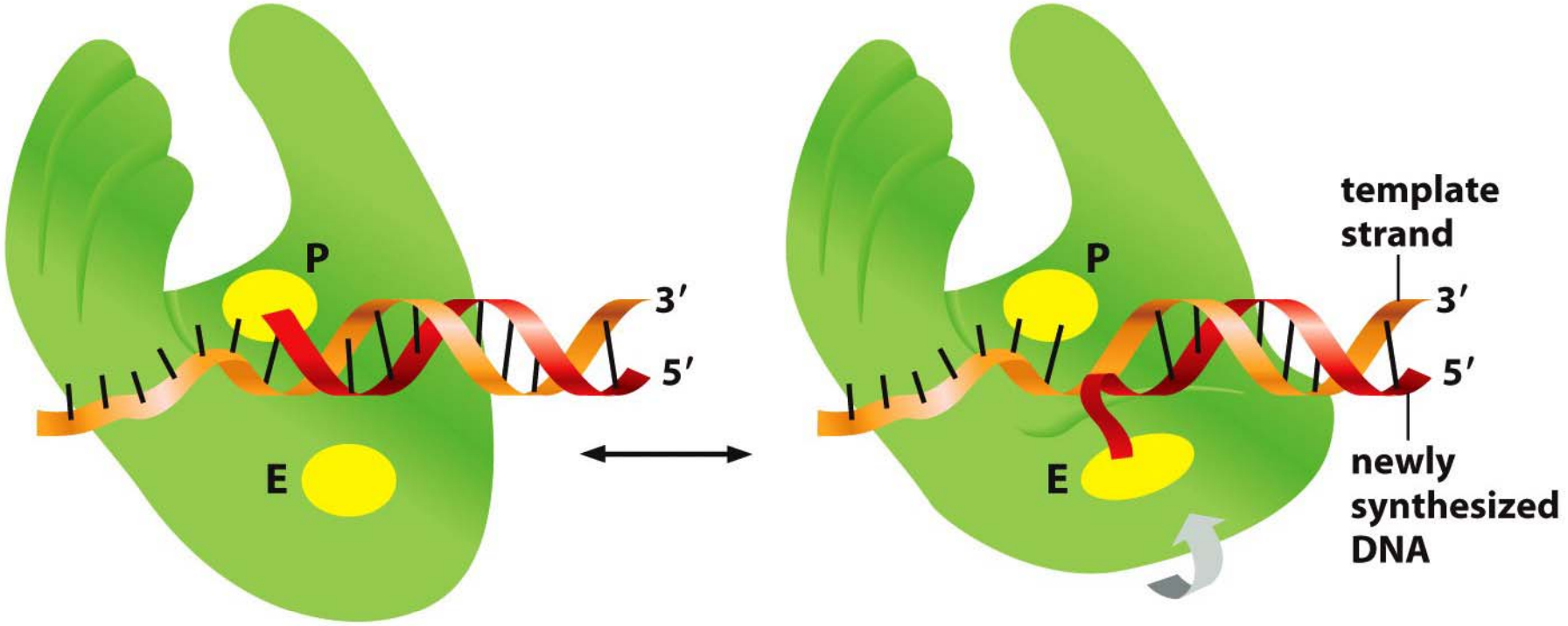
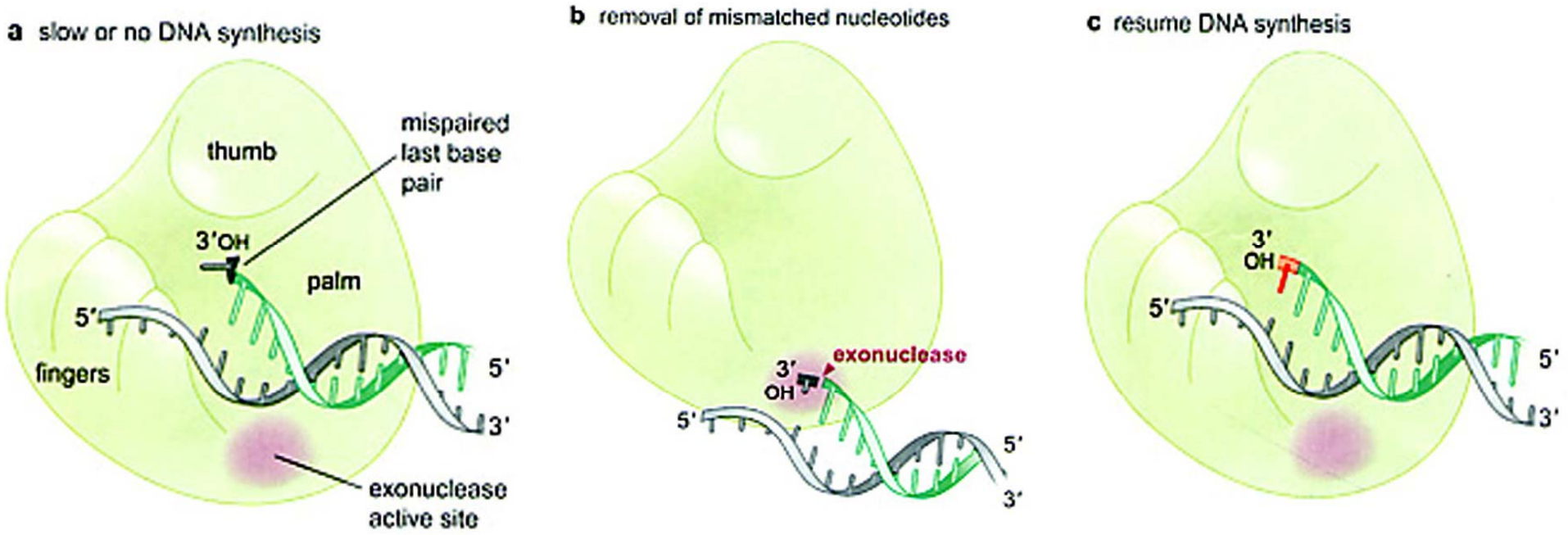


Figure 6-14 Essential Cell Biology 3/e (© Garland Science 2010)

Proofreading function of *E. coli* DNA polymerases



When an incorrect base is incorporated during DNA synthesis, the polymerase pauses, then transfers the 3′ end of the growing chain to the exonuclease site where the mispaired base is removed. Then the 3′ end flips back into the polymerase site, where this region is copied correctly.

Proofreading play a critical role in maintaining sequence fidelity during replication

- *E. coli* DNA polymerase I (3′ → 5′ exonuclease activity) and DNA polymerase III (ε subunit of the core polymerase) have proofreading activity.

- In animal cells, both of the δ and ε DNA polymerases, but not the α polymerase, have proofreading activity.

DNA的修复(DNA Repair)

由于染色体**DNA**在生命过程中占有至高无上的地位，**DNA**复制的准确性以及**DNA**日常保养中的损伤修复就有着特别重要的意义。

大肠杆菌中DNA的修复系统

DNA修复系统	功　　能
错配修复(mismatch repair)	修复错配
切除修复(碱基、核苷酸切除修复)（Base-excision repair）(nucleotide-excision repair)	切除突变的碱基和核苷酸片段
重组修复(recombinant repair)	复制后的修复，重新启动停滞的复制叉
DNA直接修复(direct repair)	修复嘧啶二体或甲基化DNA
SOS系统(细胞DNA受到损伤或复制系统受到抑制时，细胞为求生存而产生的一种应急措施)	诱导DNA损伤修复、诱变效应、细胞分裂的抑制以及溶原性细菌释放噬菌体等，导致变异

DNA复制和修复的保真度的记录保存在基因组序列中

Figure 6-28 Essential Cell Biology 3/e (© Garland Science 2010)

人与鲸性别决定基因很明显是相似的

DNA的转座
(DNA Transposition)

DNA的转座，或称移位(**transposition**)，是由可移位因子（**transposable element**）介导的遗传物质重排现象。

A **transposon (transposable element)** is a DNA sequence able to insert itself (or a copy of itself) at a new location in the genome, without having any sequence relationship with the target locus.

转座子的发现

1951年，通过对玉米籽粒色斑不稳定遗传现象，B.McClintock(美)首次提出转座子的概念。

Ds-Ac系统(Dissociation-Activation System)：

- 抑制因子（inhibitor, I)，后改称为解离因子(dissociator, Ds)。
- 激活因子(activator, Ac), 能够控制Ds因子。

Ds-Ac系统

- C 代表颜色,决定玉米糊粉层红和紫色的发生。

- 抑制因子（inhibitor, I)，后改称为解离因子(dissociator, Ds),它抑制C基因的作用。

- I和C都在玉米的第九对染色体上，且两者的座位很近。

- 激活因子(activator, Ac), 能够控制Ds因子。

•**C**与**I**基因之间易发生断裂，使**I**基因转座到原来染色体的其他部位或别的染色体上，于是**C**恢复活性表现有色。

•如果**I**（**Ds**）因子停留在原来的座位，并未发生断裂和转座，那么玉米籽粒为无色。

为什么**Ds**既能停留在原位，对**C**基因起着抑制作用，又能在玉米籽粒发育的不同阶段发生断裂和转座呢？

⇩

Ds的作用还受到激活因子***Ac***的控制

- **Ac**和**Ds**在不同的染色体上
- 没有**Ac**的情况下籽粒为无色，但当**Ac**从**1**个增加到**2**或**3**个时，籽粒上带色斑点反而减少了。说明**Ac**的增加推迟了**Ds**因子的解离和转座。
- **Ds**和**Ac**都可以转座：**Ac**的作用是自主的，而**Ds**的行为却依赖于**Ac**的作用。

转座作用的遗传学效应

①转座引起插入突变,导致结构基因失活。

②转座产生新的基因。

③转座产生的染色体畸变。

④转座引起生物进化。

SNP的理论和应用

单核苷酸多态性

(SNP, Single Nucleotide Polymorphism)

指基因组DNA序列中由于**单个核苷酸**（A，T，C和G）**的突变**而引起的多态性。

SNP是基因组中**最简单最常见**的多态性形式，具有**很高的遗传稳定性**。

SNP检测和分析技术成为**第三代遗传标记**。

SNP概述

一个SNP表示在基因组某个位点上一个核苷酸的变化：

- 转换（C←→T，在其互补链上则为G←→A）
  占SNP总量的2/3
- 颠换（C←→A，G←→T，C←→G，A←→T）

SNP广泛存在于人类基因组中，其发生频率约为1%或更高。一个人类个体大约携带300万-1000万个SNPs。

根据SNP在基因组中的分布位置可分为三类

1. 基因编码区SNP（cSNP），20%

（1）同义cSNP（synonymous cSNP）

即SNP所导致的编码序列改变并不影响其所翻译的蛋白质的氨基酸序列，突变碱基与未突变碱基的含义相同；

（2）非同义cSNP（non-synonymous cSNP）

指碱基序列的改变可使以其为蓝本翻译的蛋白质序列发生改变，从而影响了蛋白质的功能。是导致生物性状改变的直接原因。

2. 基因调控区SNP（pSNP）

影响基因表达量的多少

3. 基因间随机非编码区SNP（rSNP）

SNP的应用

1. 人类单倍型图的绘制

已有超过150万个SNP被精确定位于各染色体上。

将为我们精确定位复杂疾病，如糖尿病，癌症，心脏病，中风，哮喘等易感基因提供重要信息。

2．SNP与疾病易感基因的相关性分析

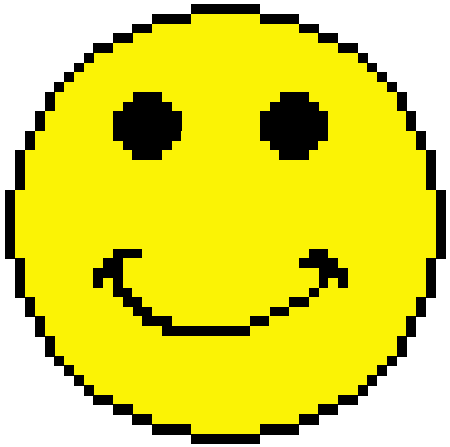
SNP作为新一代遗传标记在人类疾病研究中显示出极高的潜在价值。已经发现了高血压、哮喘、类风湿关节炎、肺癌、前列腺癌等许多易感基因。

3．指导用药与药物设计

通过研究SNP与个体对药物敏感或耐受的相关性研究，可能阐明遗传因素对药效的影响，因此可能建立与基因型相关的治疗方案，对病人施行个性化用药。另外，随着SNP的研究与药物基因组学的结合，根据特定的基因型来设计药物将成为可能。

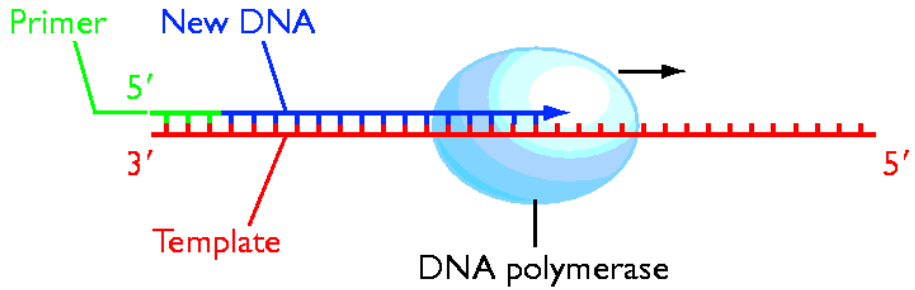
思考题

1. 染色体具备哪些作为遗传物质的特征？
2. 什么是核小体？简述其形成过程。
3. 简述真核生物染色体的组成，它们是如何组装的？
4. 简述DNA的一、二、三级结构。
5. 原核生物DNA具有哪些不同于真核生物DNA的特征？
6. 简述DNA双螺旋结构及其在现代分子生物学发展中的意义。
7. DNA复制通常采取哪些方式？
8. 简述原核生物DNA的复制特点。
9. 真核生物DNA的复制在哪些水平上受到调控？
10. 细胞通过哪几种修复系统对DNA损伤进行修复？
11. 什么是转座子？可分为哪些种类？
12. 请说说插入序列与复合型转座子之间的异同。
13. 组蛋白上都存在哪些修饰？其作用是什么？



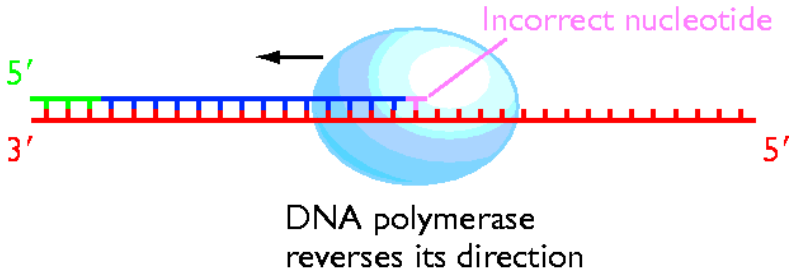
Thank you!

(A) 5′→3′ DNA synthesis



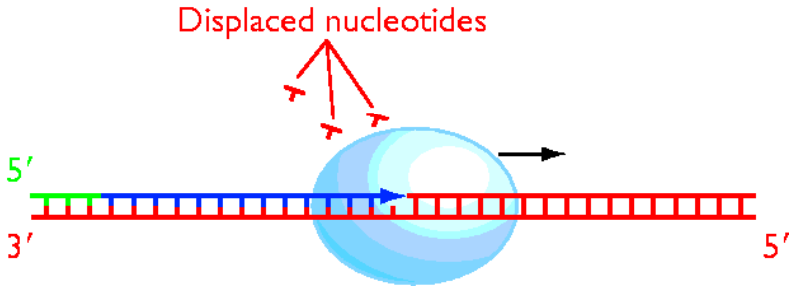
DNA聚合酶III

(B) 3′→5′ exonuclease activity



DNA聚合酶I、II、III
(a proofreading activity)

(C) 5′→3′ exonuclease activity



DNA聚合酶I
(DNA损伤的修复）

端粒使真核生物染色体末端完成DNA合成

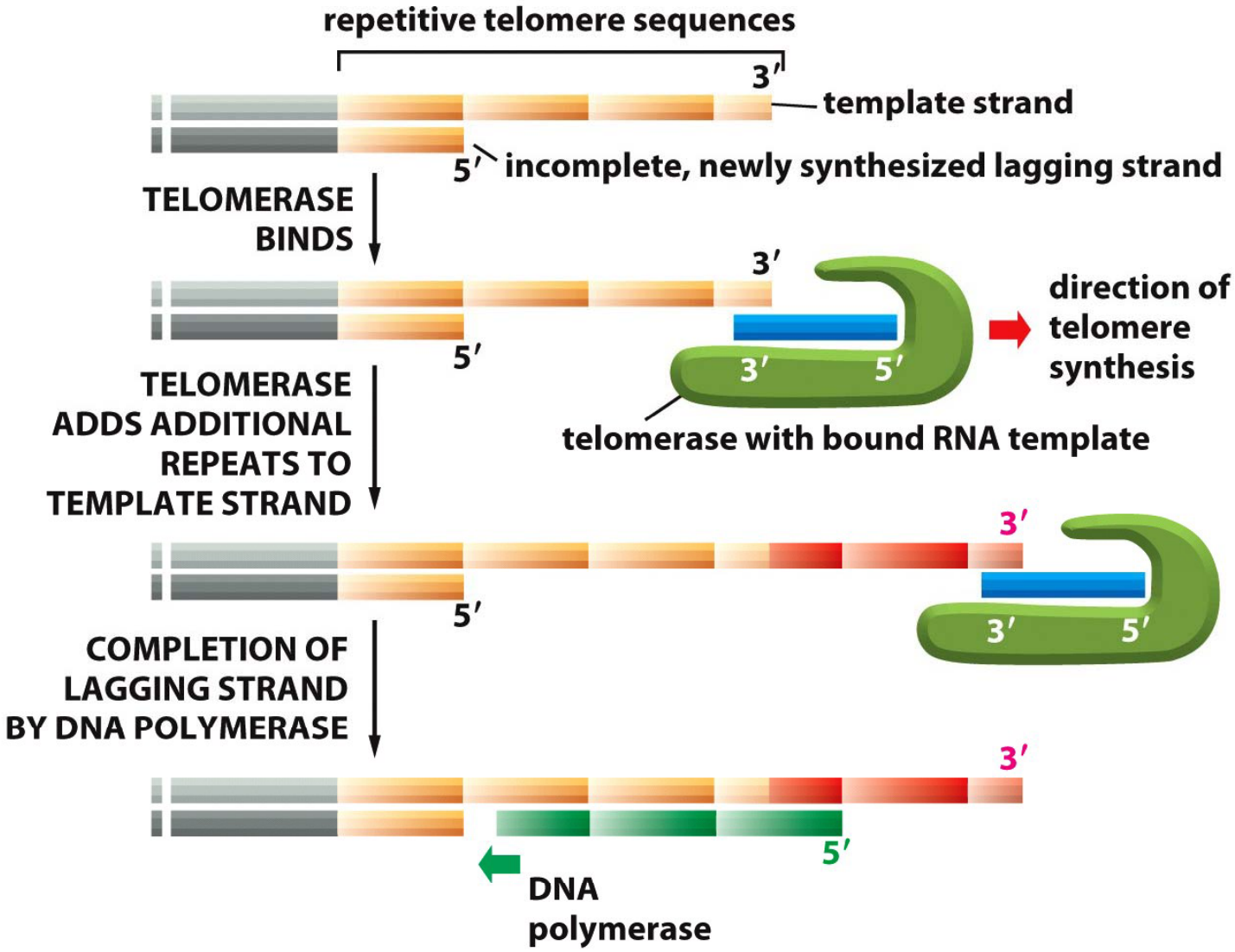


Figure 6-18 Essential Cell Biology 3/e (© Garland Science 2010)