

(来源：不详)

第3章

DNA复制

(DNA Replication)

- 3.1. 复制起点与方向**
- 3.2. Semi-Conserved Replication**
- 3.3. 复制的方式**
- 3.4. 线状 DNA 的复制**
- 3.5. DNA 复制的相关调控与修饰**

基本概念

(Basic Concept)



遗传物质的分子基础 → 分子生物学的核心

Watson & Crick: 一种遗传物质，必须能行使两种功能，即自我复制和对细胞的高度特异性的影响

遗传物质的基本属性：**基因的自我复制**

基因的突变

控制性状的表达

DNA复制

亲代双链DNA分子在DNA聚合酶的作用下，分别以每单链DNA分子为模板，聚合与自身碱基可以互补配对的游离的dNTP，合成出两条与亲代DNA分子完全相同的子代DNA分子的过程。

- Replicon;

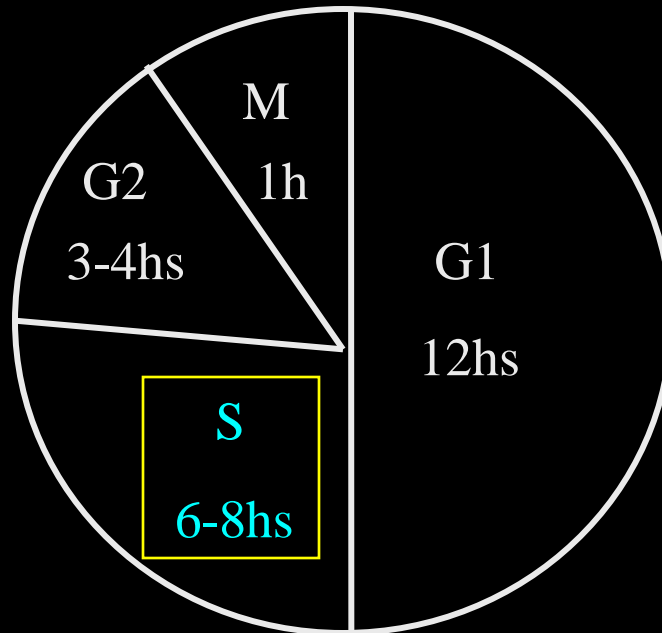
A unit of the genome in which DNA contain a region from origin to terminator

- Replisome;

The multi protein (30±) structure that assembles at replicating fork to undertake synthesis of DNA

DNA replication at phase S of cell cycle

E.coli 37 °C
0.5 h
10⁵ bp/min



mammalian cell
22-25hrs
500-5000 bp/min



• 复制机理的复杂性

D.S. DNA \longrightarrow S.S. DNA 能量的供求

构型的变化 超螺旋 \longrightarrow 线状, 开环状

多种酶类的互动 \longrightarrow **Replisome**

复制的准确性 (修复, 校正)

研究试材的特殊性 (温度敏感型ts, 突变抑制体系Su)

DNA复制速度 (E.coli 10^5 bp/min, 高速解旋 112 km/h ?)

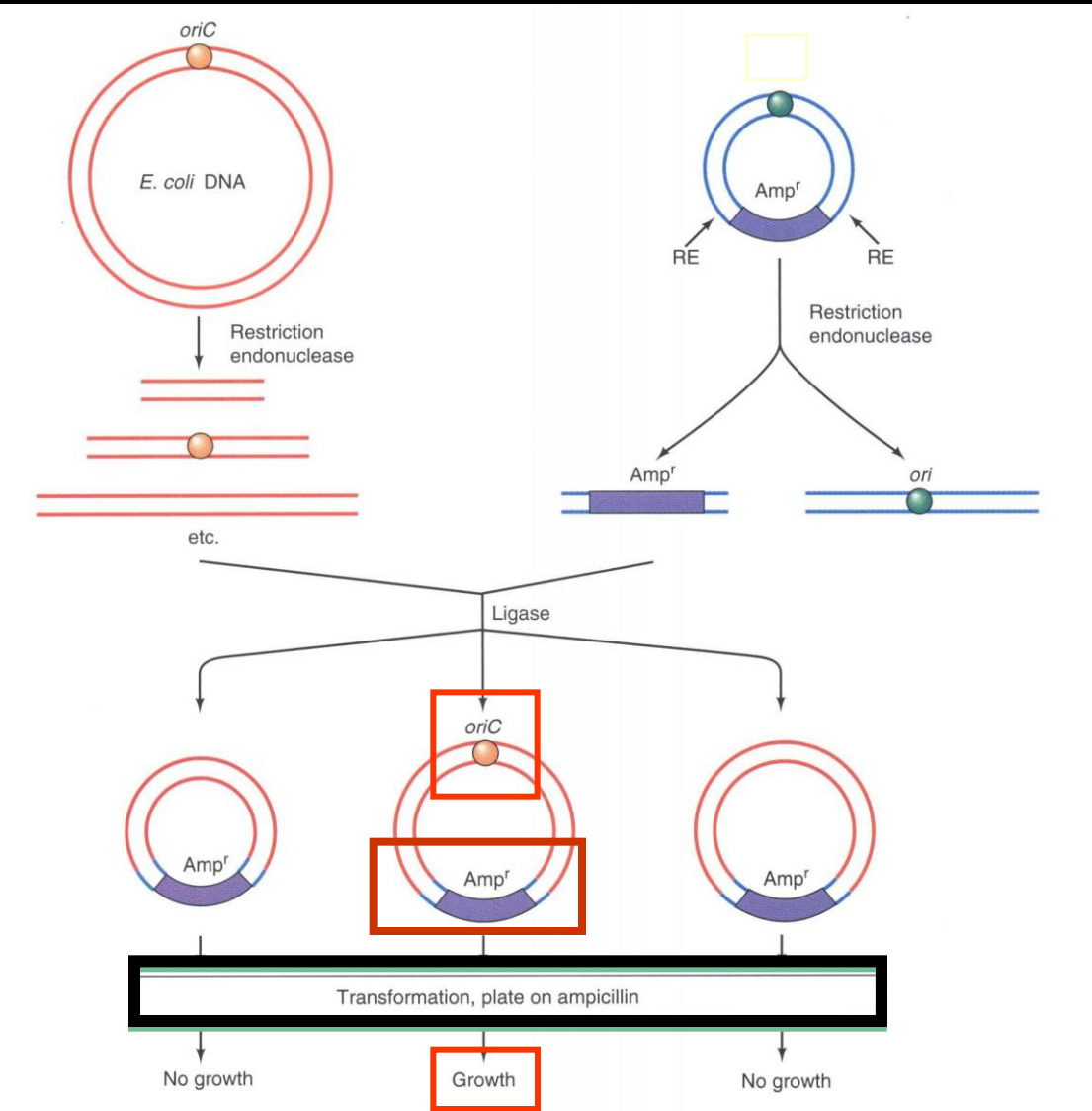
缺乏统一的模式 (D.S. DNA, S.S. DNA, Linear DNA....)

DNA复制起始控制机理知之甚少

3.1. 复制起点与方向

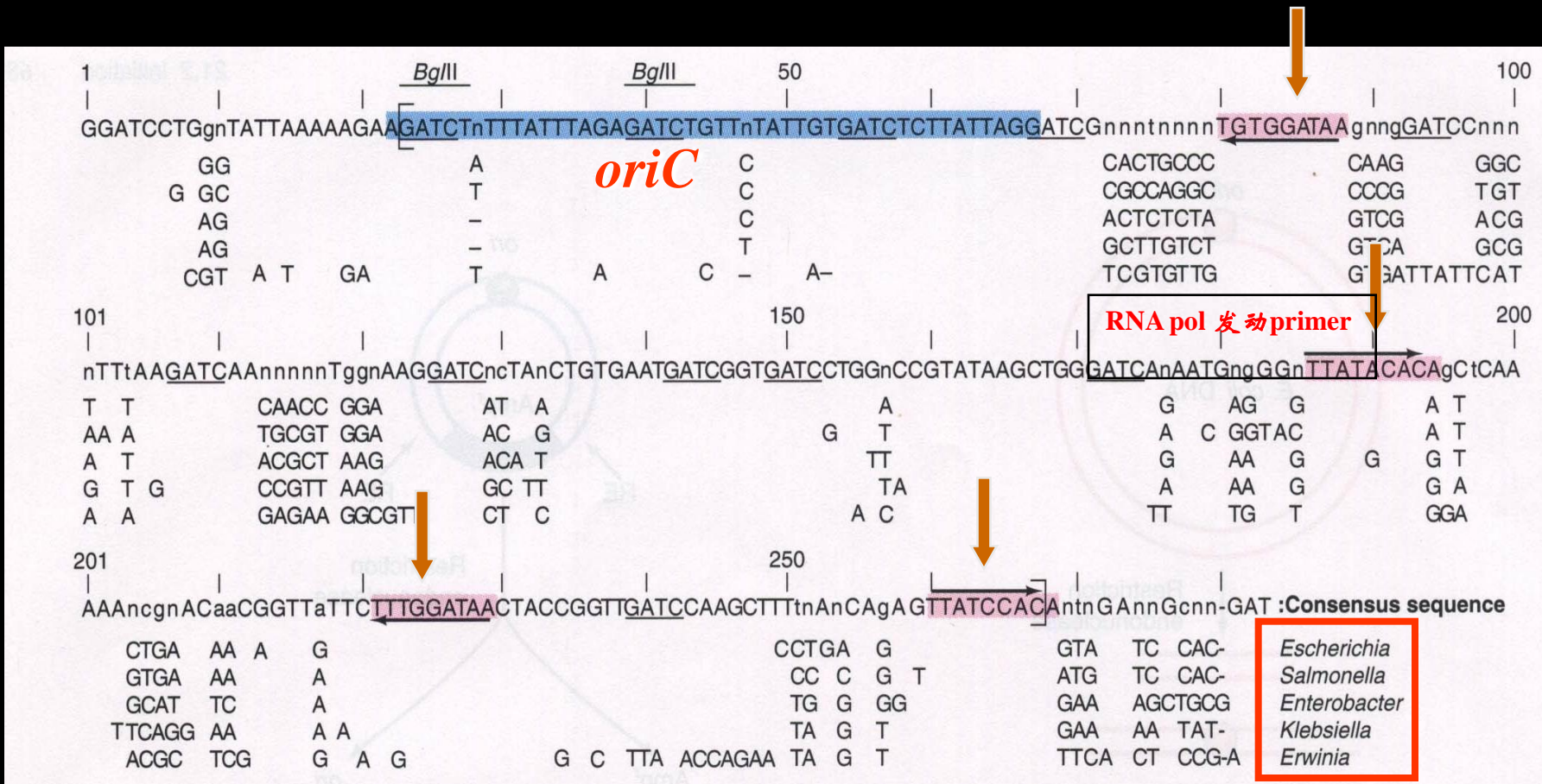
(replication origin & direction)

isolation of *oriC*

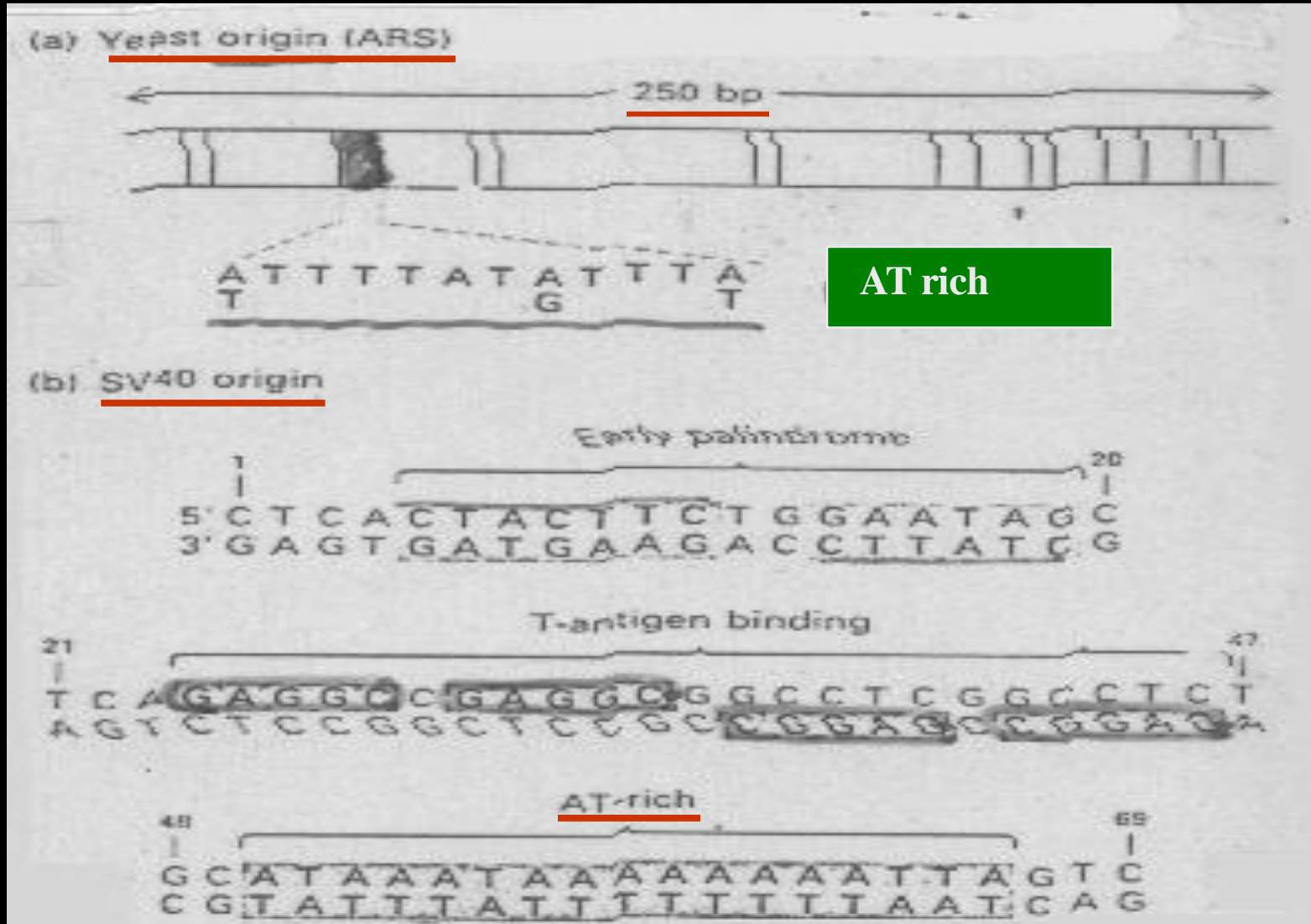


复制起点的特征

- 245bp minimal origin of replication rich AT & DNA polymerase (DnaA) binding site



真核生物复制起点的研究是以酵母为基础的

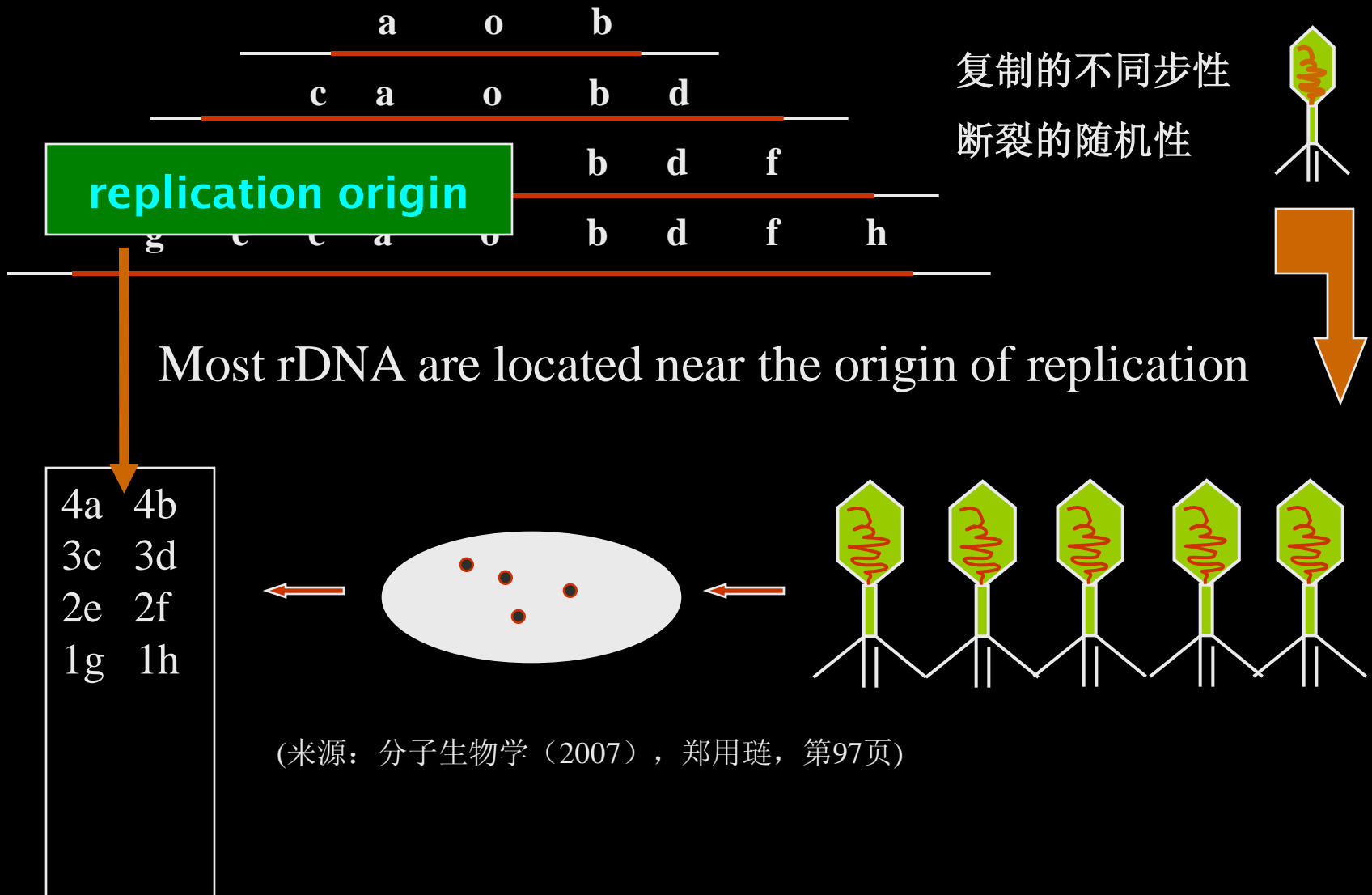


- “呼吸现象”

DNA复制原点处氢键迅速断裂与再生，
导致两条DNA链不断解链与聚合，
形成瞬间的单泡状结构的过程。

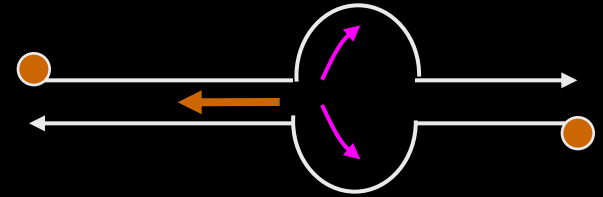
在富含AT的区域内尤为明显

• replication origin 两侧基因的转导频率高

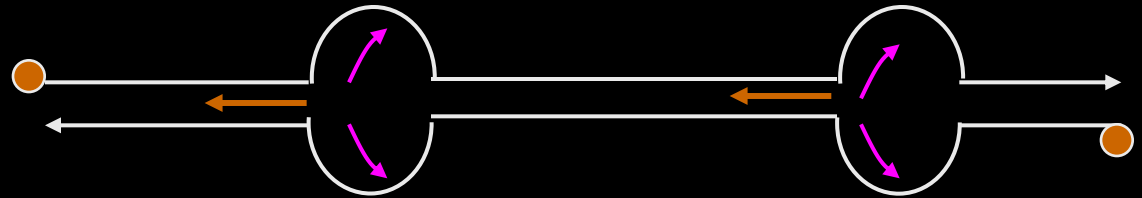


• 复制的多模式

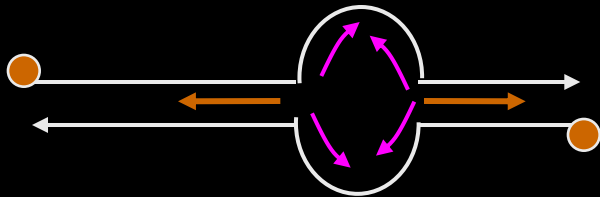
单起点、单方向



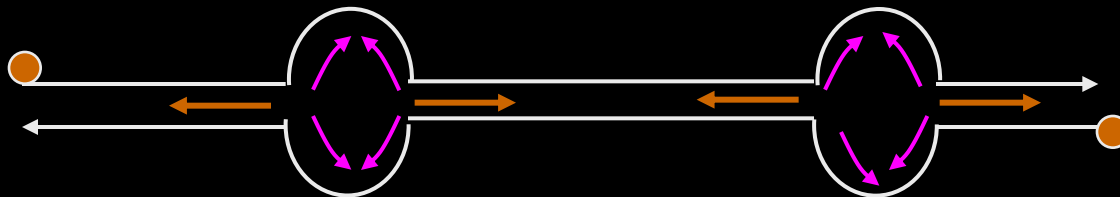
多起点、单方向



单起点、双方向



多起点、双方向



复制多模式的证据

1963 Cairns

E. coli [**thy⁻ slow-stop mut.^{ts}**]

复制发动温度敏感突变型

42°C不能发动DNA复制、但可完成DNA延伸



37 °C, **5** ci / mM **H³-T**, 6min



37 °C, **52** ci / mM **H³-T**, 6min

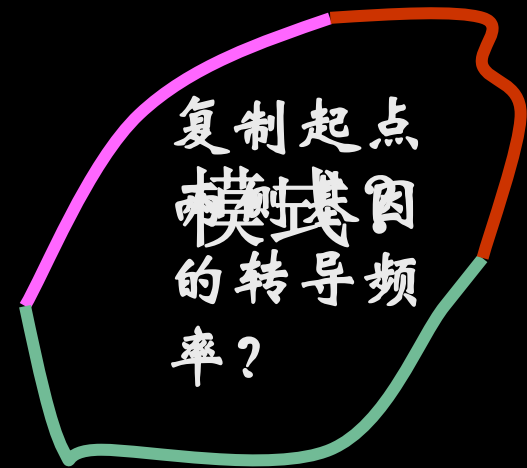


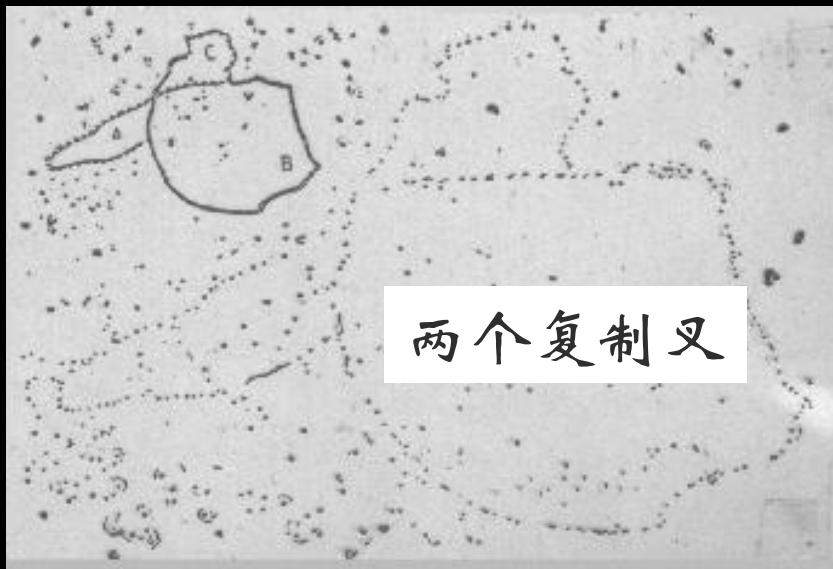
42 °C, **T**, one circle

快停突变
慢停突变

(fast-stop mutation)
(slow-stop mutation)

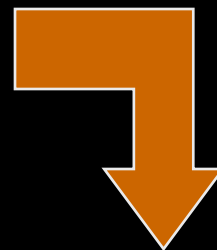
当温度升高，复制停滞在~~一半~~? 周期开始



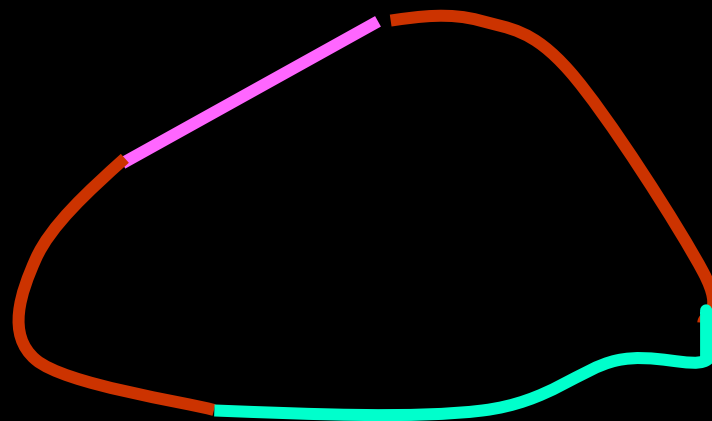


两个复制叉

(来源：不详)

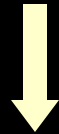


单起点、双方向



R. L. Rodriguez

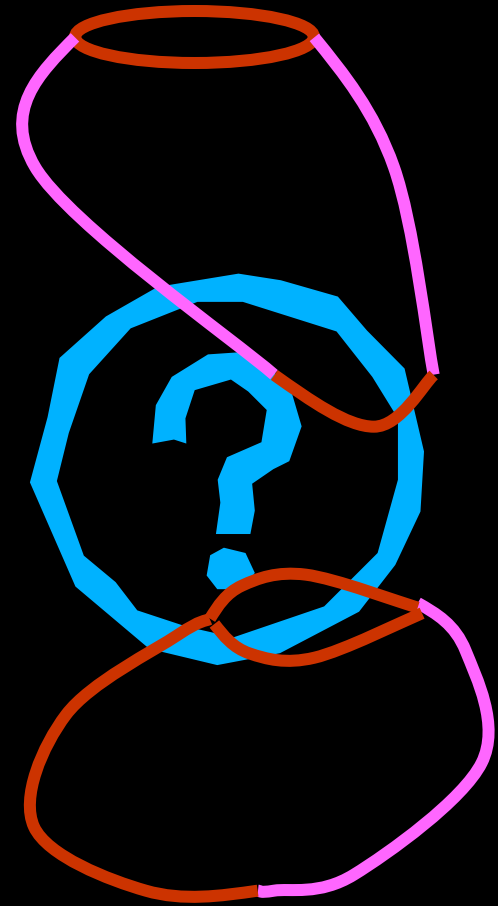
E. coli thy- slow-stop mut.^{ts} 42°C



37°C, 5 ci / mM H³-T, 20min



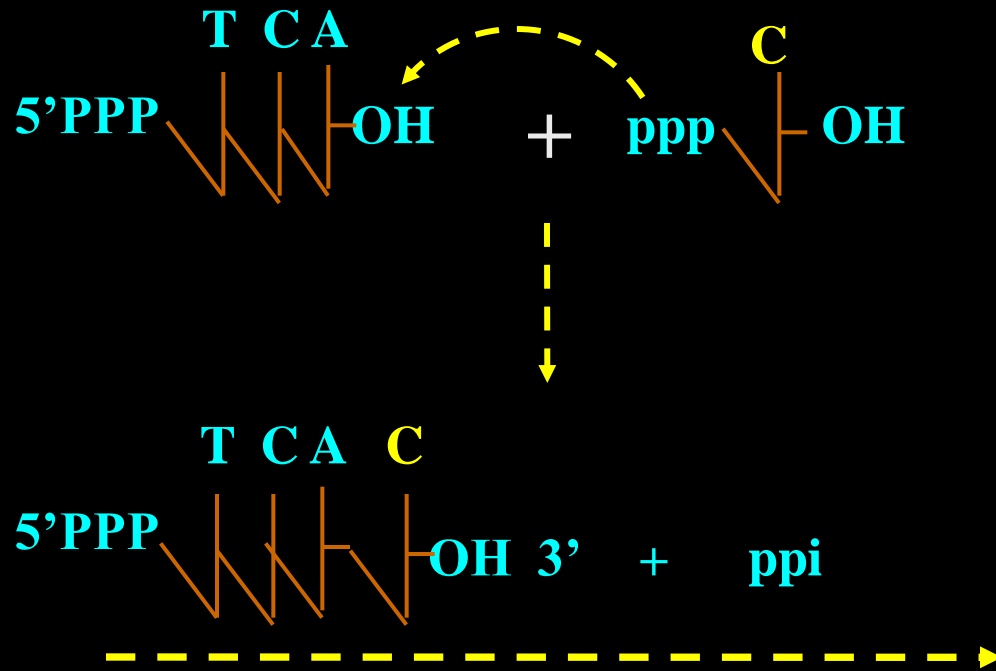
37°C, 52 ci / mM H³-T, 45min



- 子链DNA延伸方向

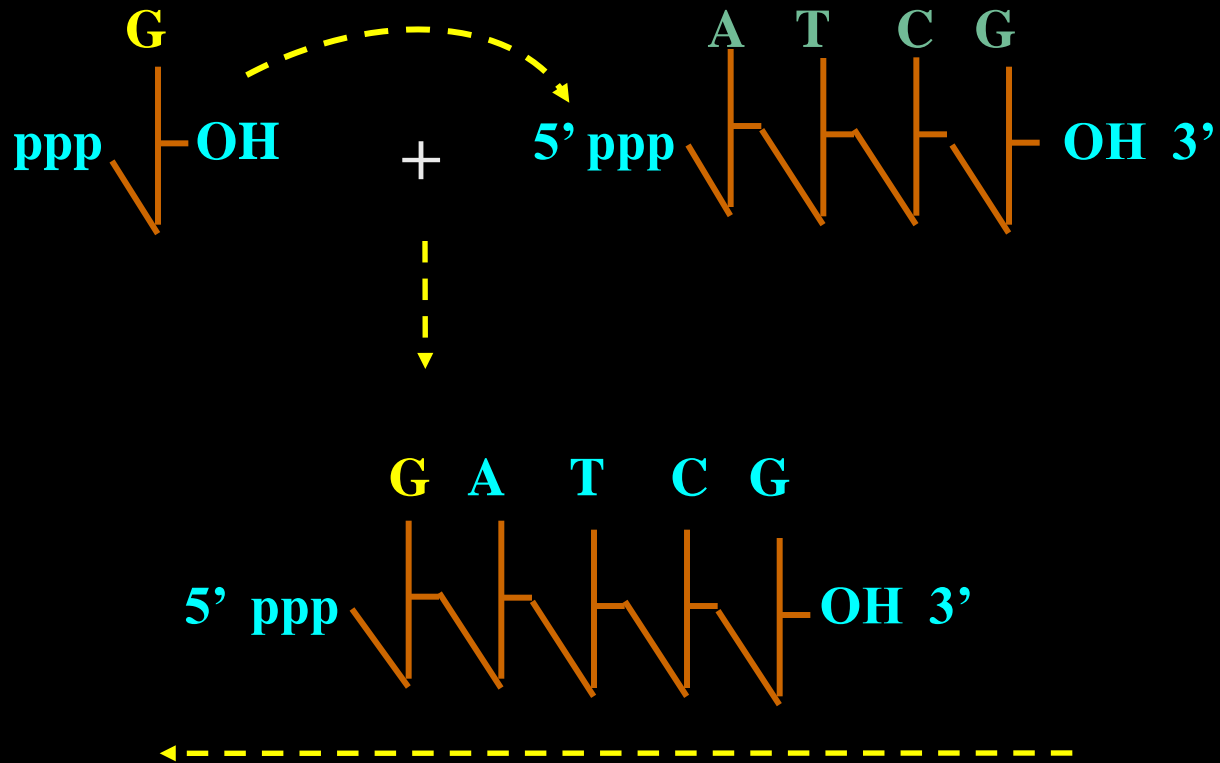
DNA polymerase reacts on the 3' end **only**

New DNA elongation from 5' to 3' direction



进化中保留
的深刻的、
选择与适应
的、化学及
功能的根源

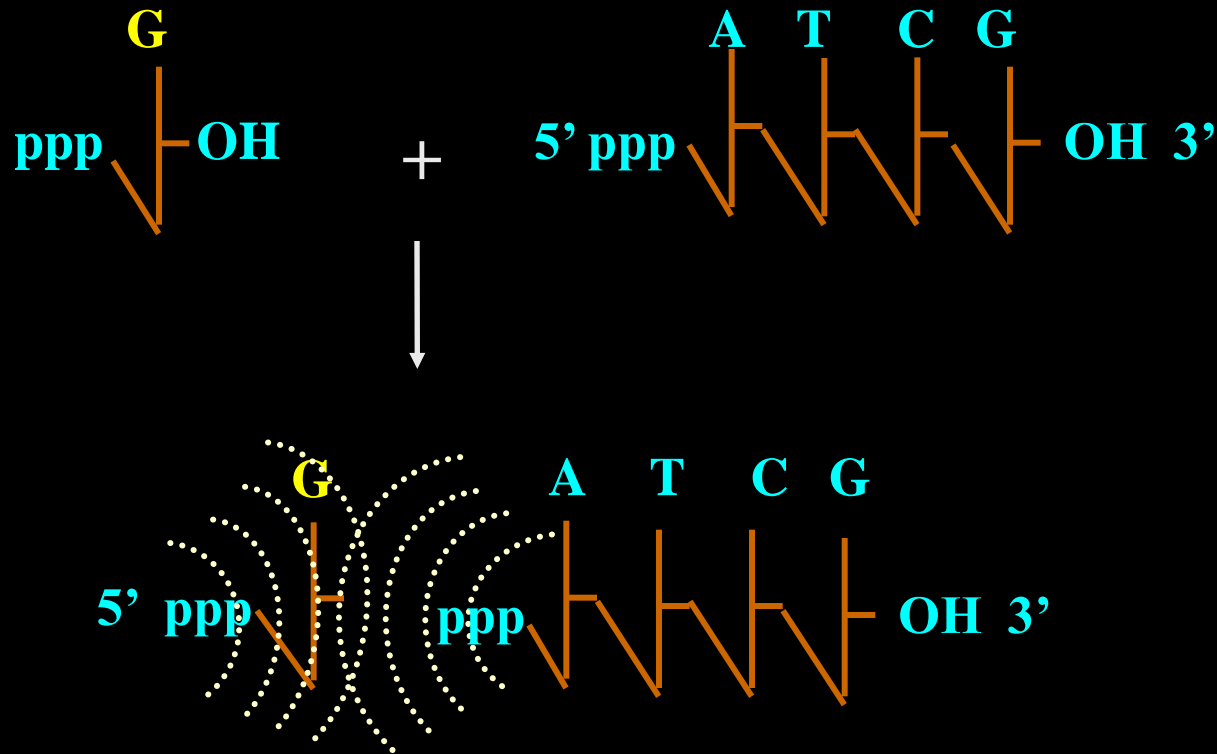
如果DNA的延伸方向是 3' → 5'



游离dNTP具有ppp

因能量的需要，

DNA的5'端必须带有PPP

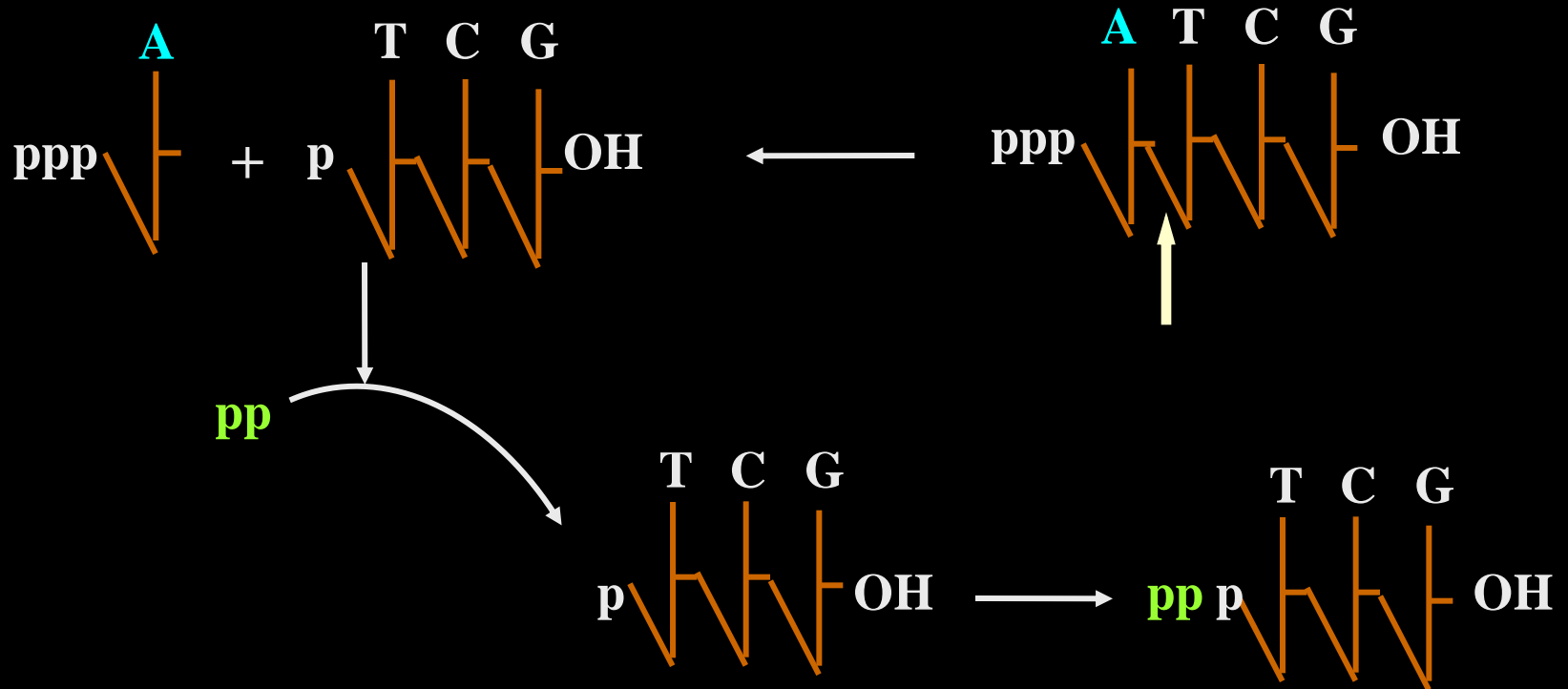


在0.2M NaCl 的生理环境中，磷酸基团间的强电负性，使dNTP难以聚合到

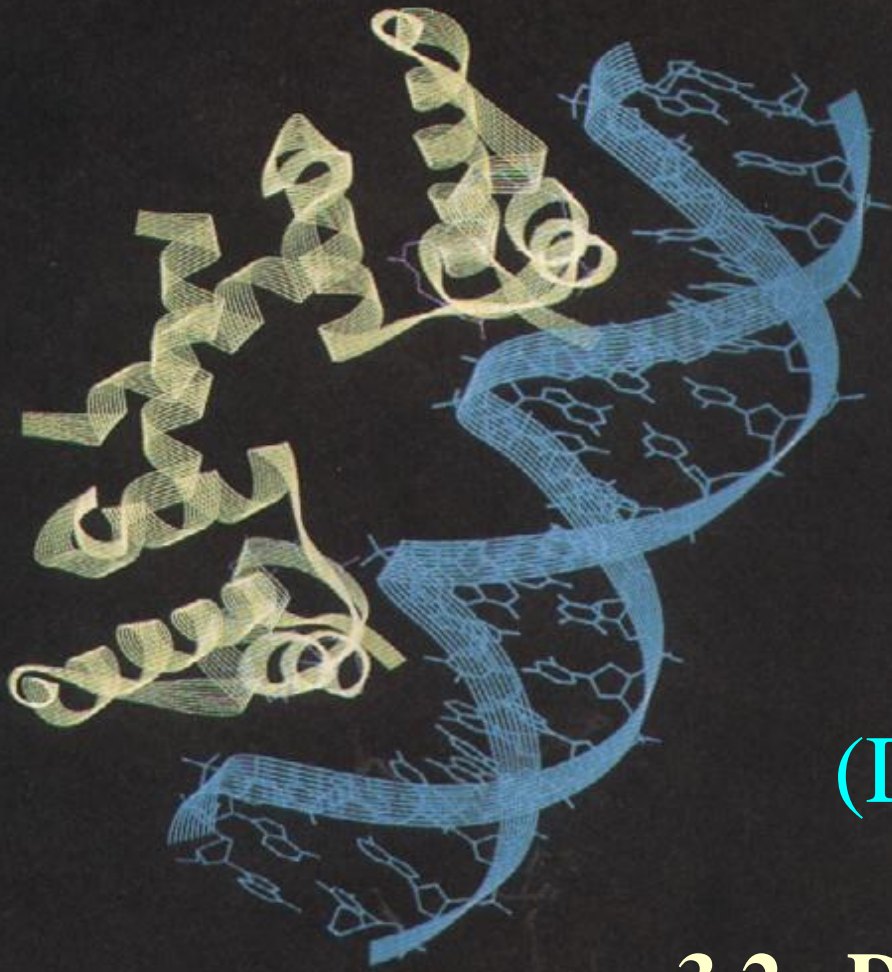
DNA的5'端，而且双链DNA的5'端碱基配对困难

需要其他机制以解脱

碱基发生错配后的校正.....



费时、费能、增加脱磷酸、加磷酸的能量消耗



第3章

DNA复制

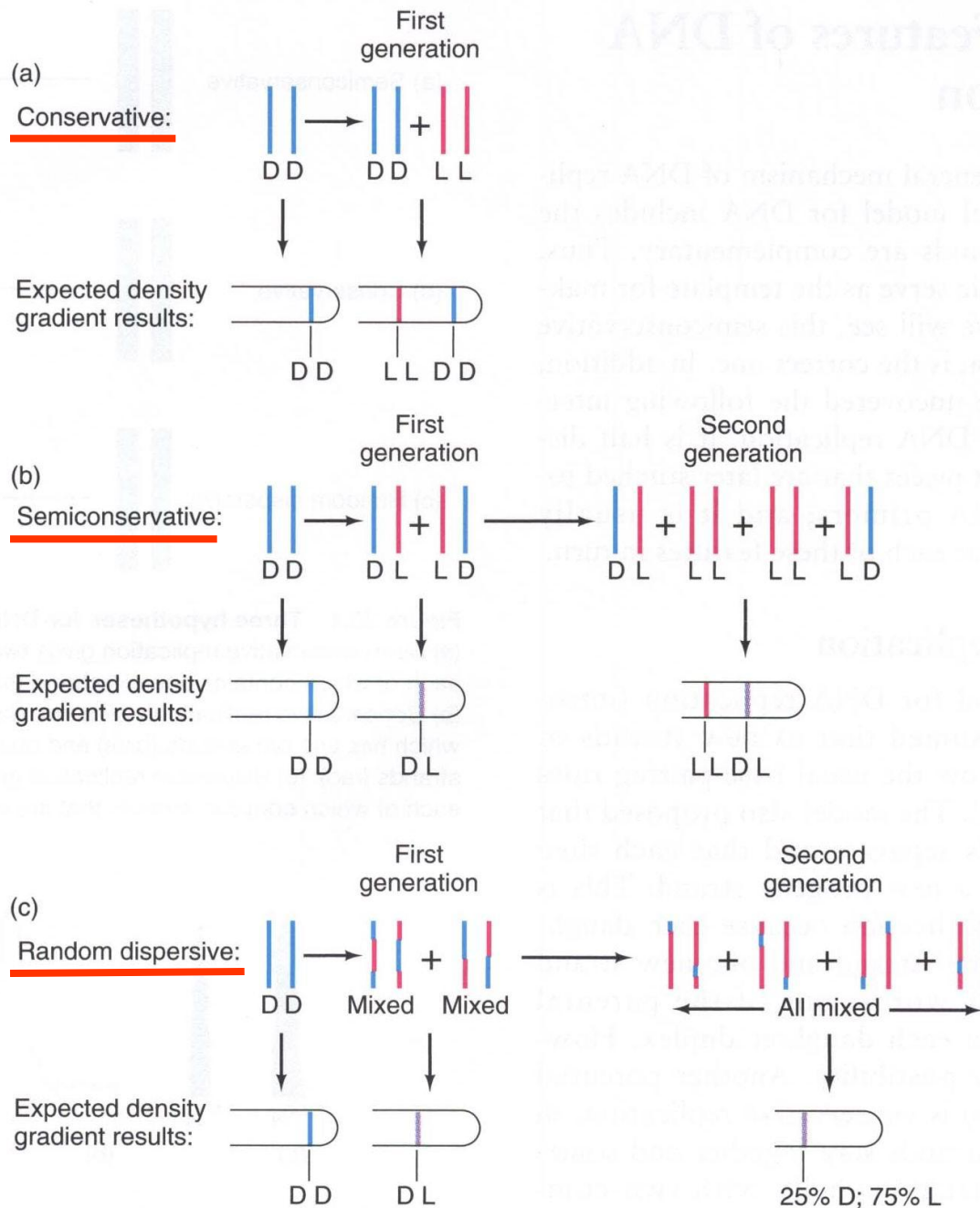
(DNA Replication)

3.2. DNA的半保留复制

(Semi-Conservation Replication)

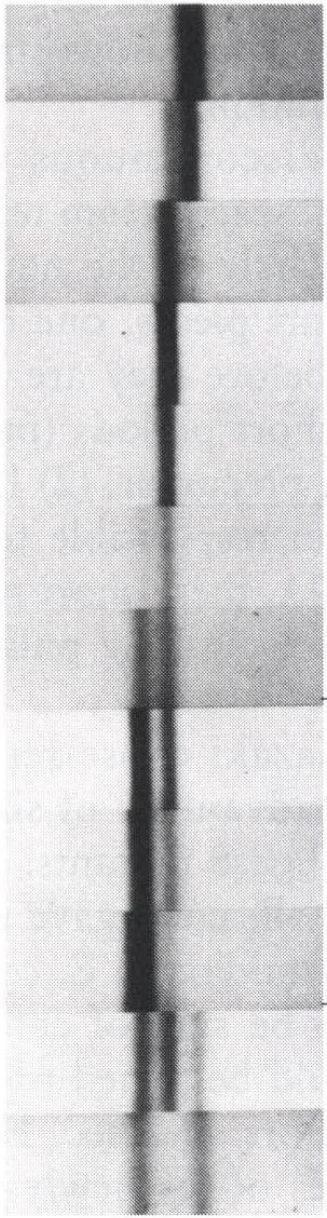
(来源: 不详)

Three replication hypotheses



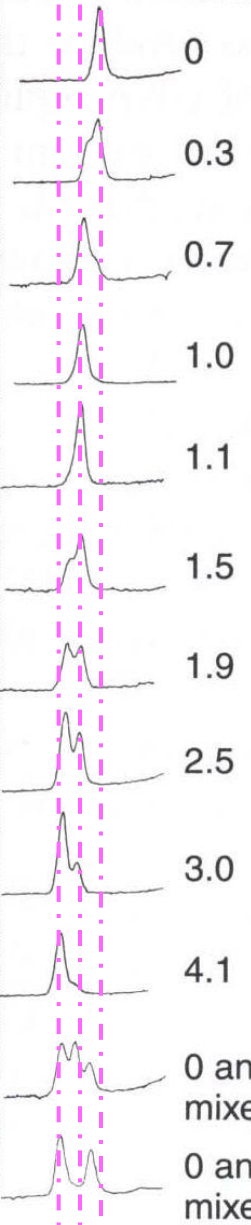
(Source: Molecular Biology(2002), Robert F. Weaver, Page 641)

(a)



(b)

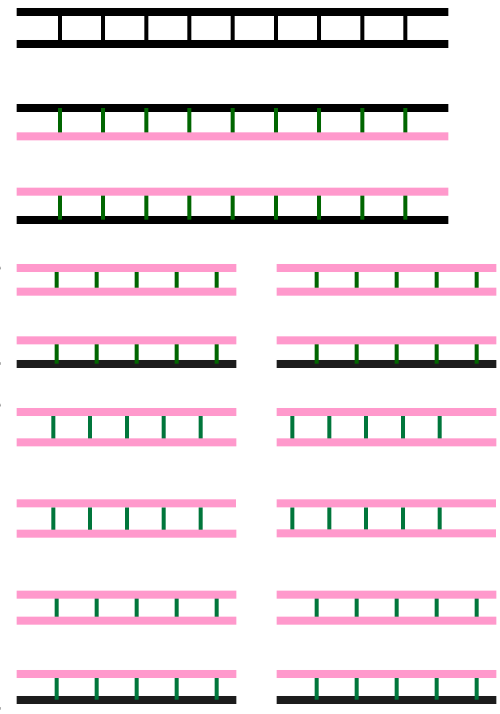
Generations (CsCl gradient centrifuge)



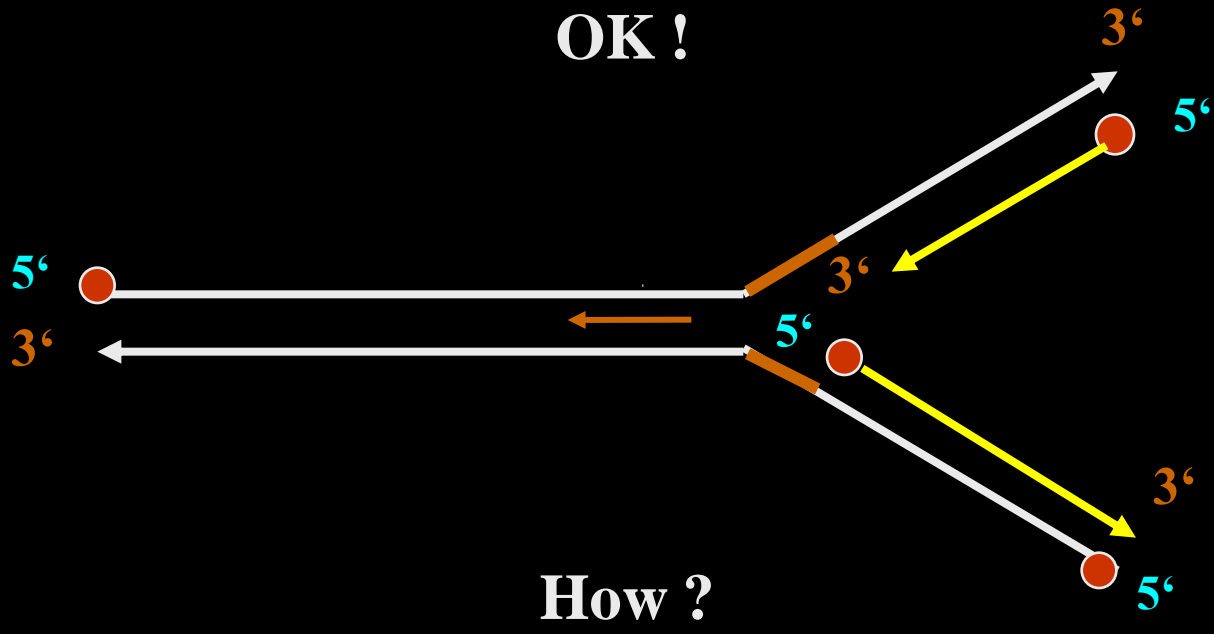
N15

N14

DNA



Semi-Conservation Replication



a) **Okazaki fragment** 1968 Reiji Okazaki



E.coli [**t**]

2", 7", 15", 60"

20°C in **dT-H³** 60'

pulse-labeling in **dT-H³**

pulse-chase

stop in KCN 0°C

transfer to dT then continue

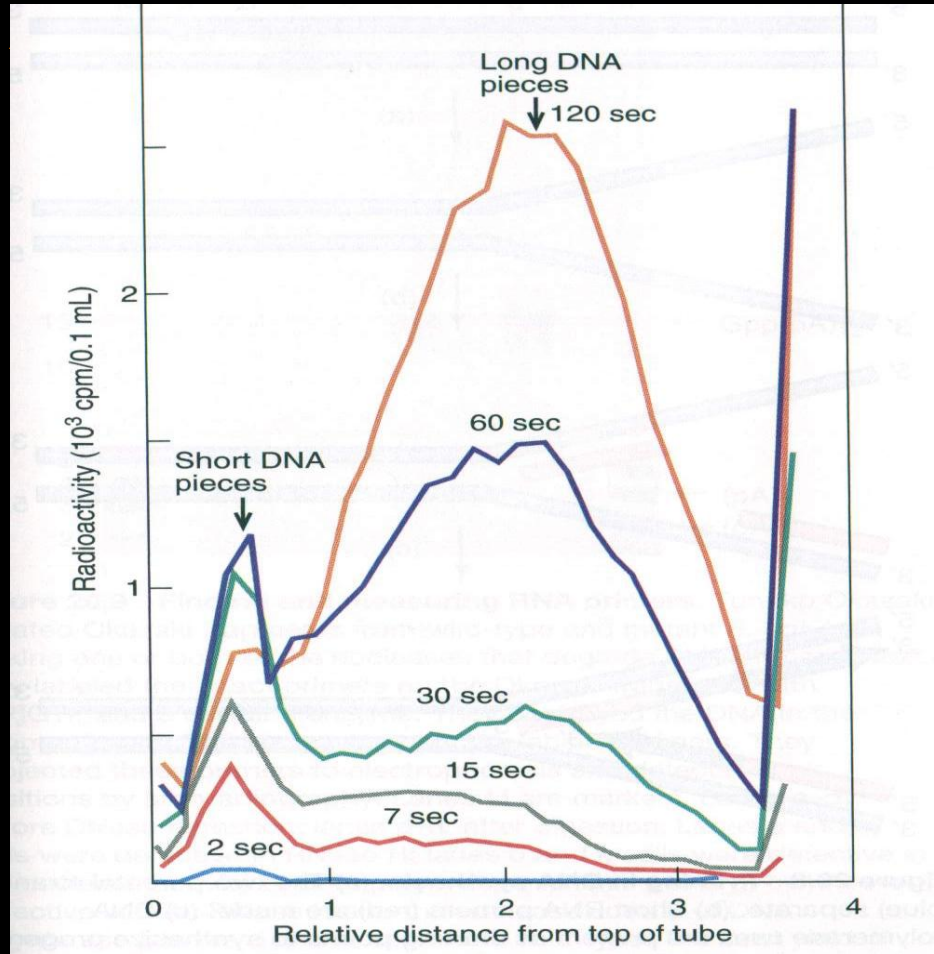
D.S. DNA

S.S. DNA

Density gradient of sucrose

Measure **H³-T**

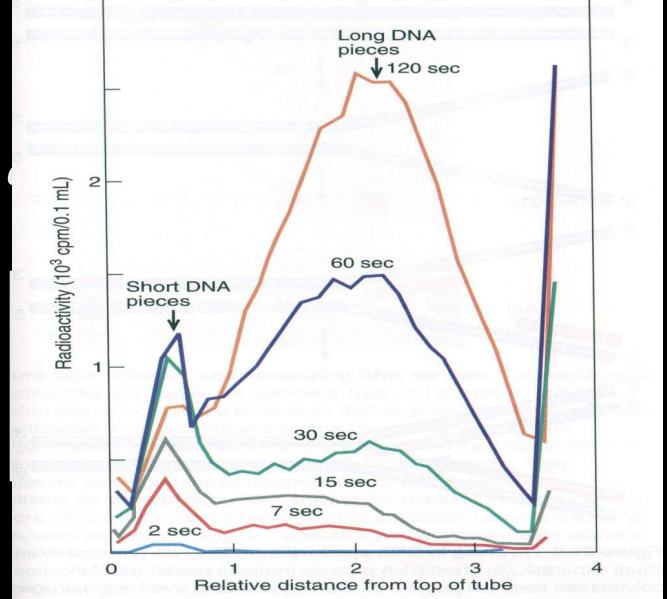
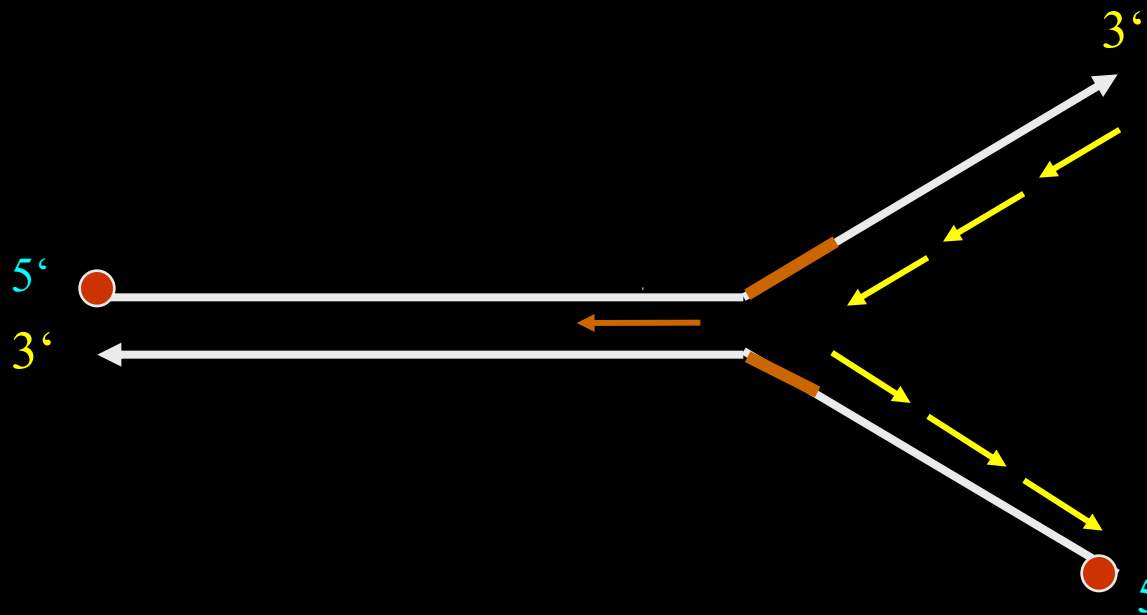
H³-T



(Prok. 400Nt/sec)

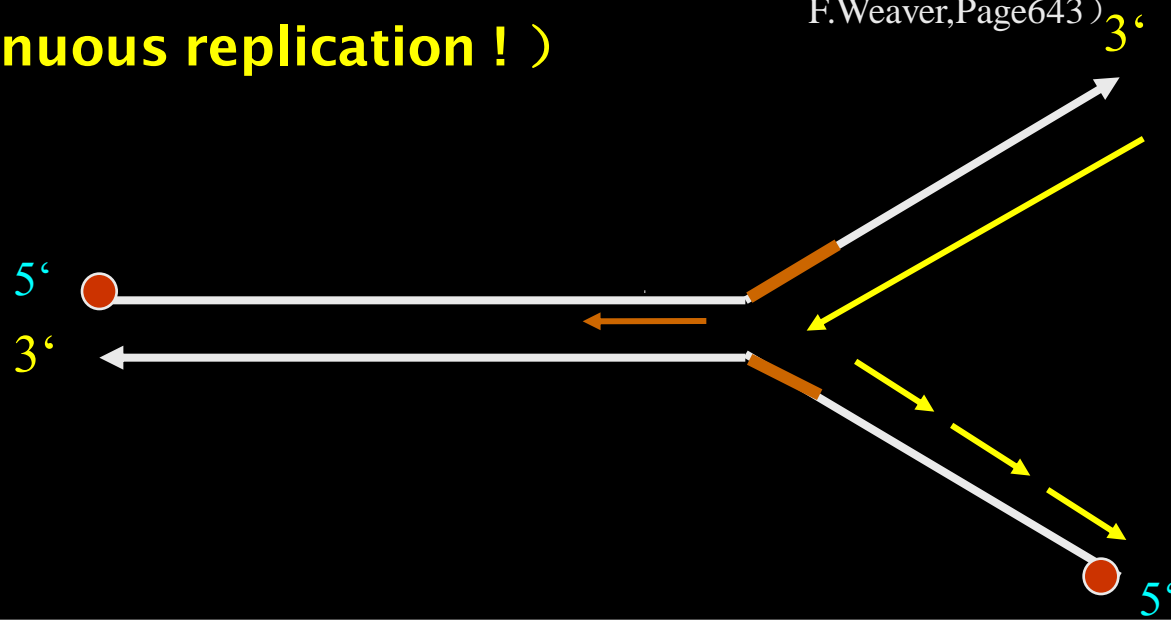
DNA replication in Okazaki fragment 1kb

(Source:Molecular Biology(2002),Robert F.Weaver,Page643)



(Source: Molecular Biology (2002), Robert F. Weaver, Page 643)

(semi-discontinuous replication !)



At least one strand of DNA replication in Okazaki fragment 1kb

a) 半不连续复制

(semi-discontinuous replication)

证据

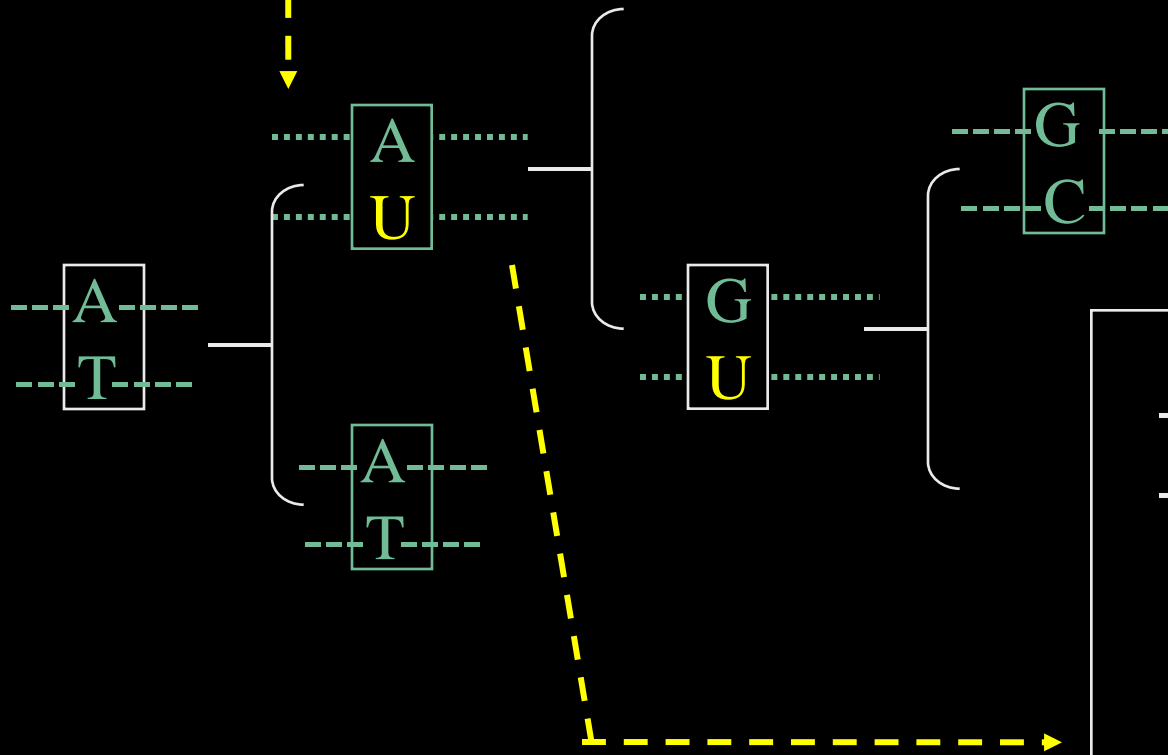
- dump fragment in DNA

dUTP : dTTP = 1 : 300 in cell

DNA 中不能有U?

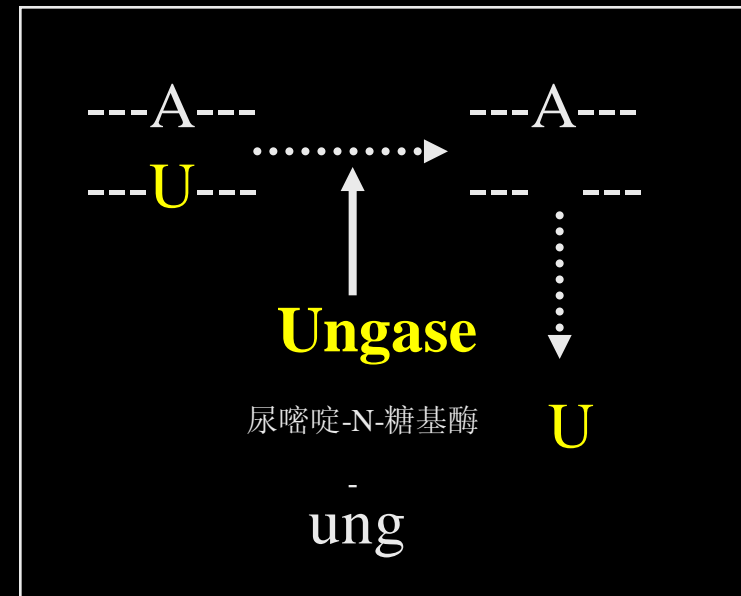
少数dUTP

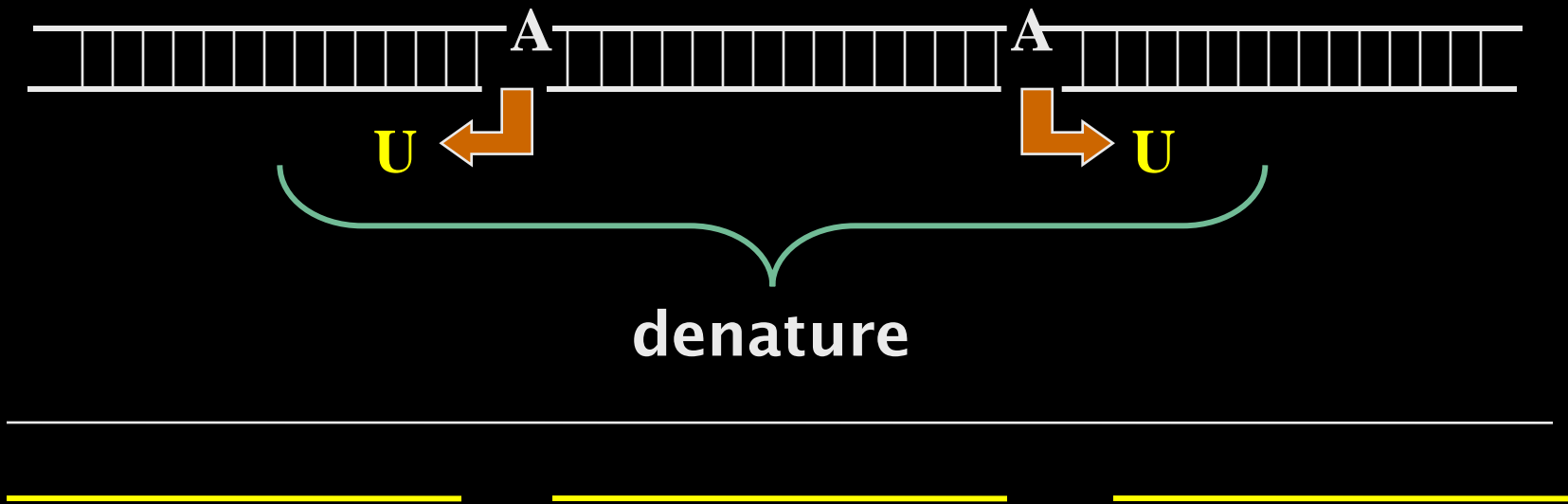
dUTPase ← dut gene



突变频率 =

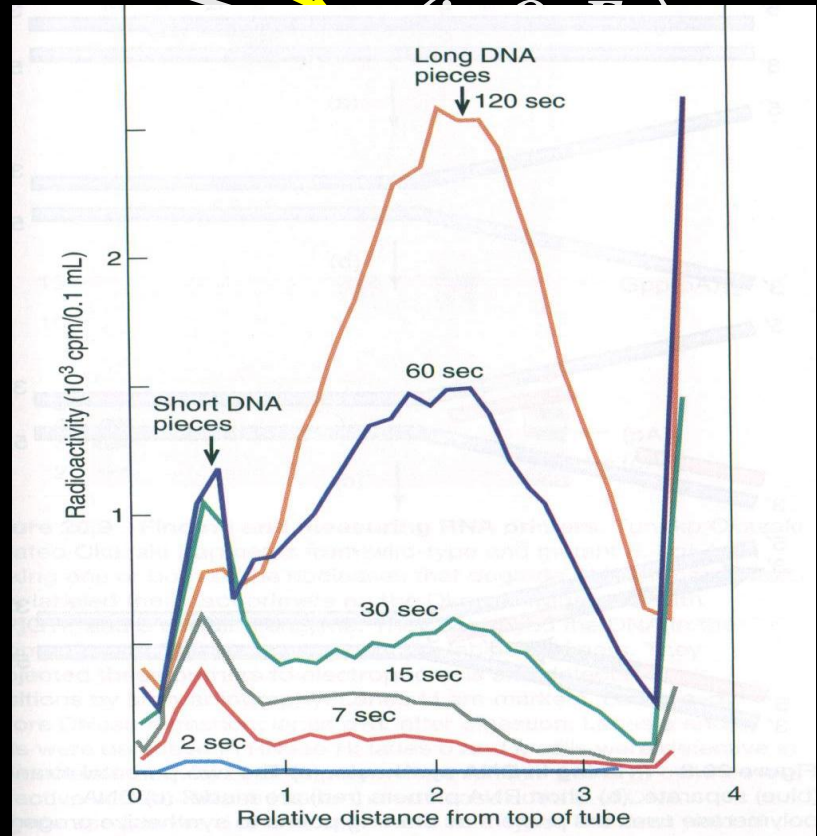
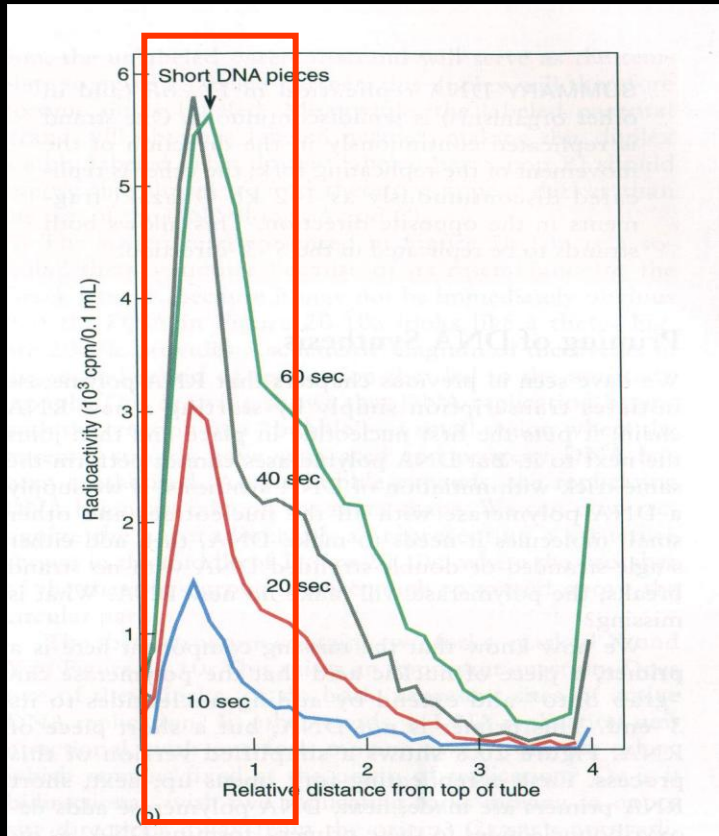
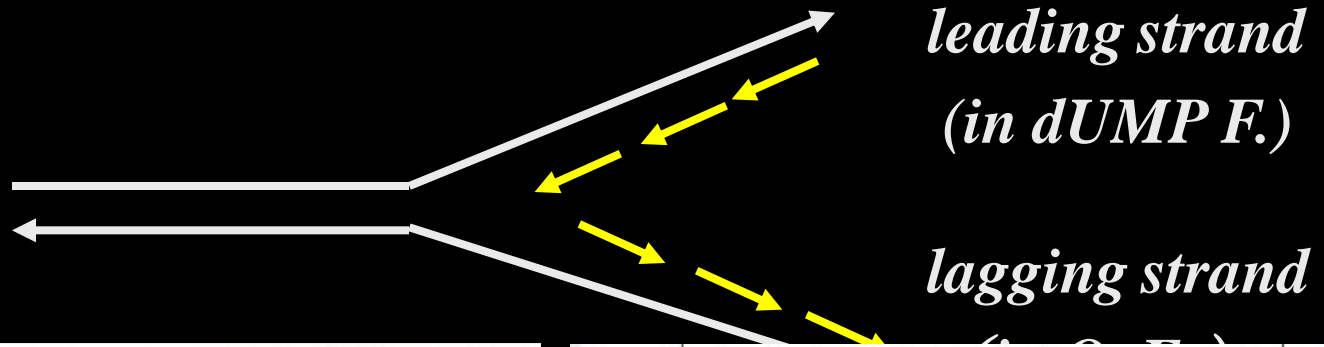
1/1200 ! ?





dump fragment ~ 1200base ~ Okazaki fragment

- ung^{-} → dump fragment longer
- dut^{-} → dump fragment shorter



(Source: Molecular Biology(2002), Robert F. Weaver, Page 643)

DNA semi-discontinuous replication

leading strand , lagging strand 均有 **dUMP** 的掺入

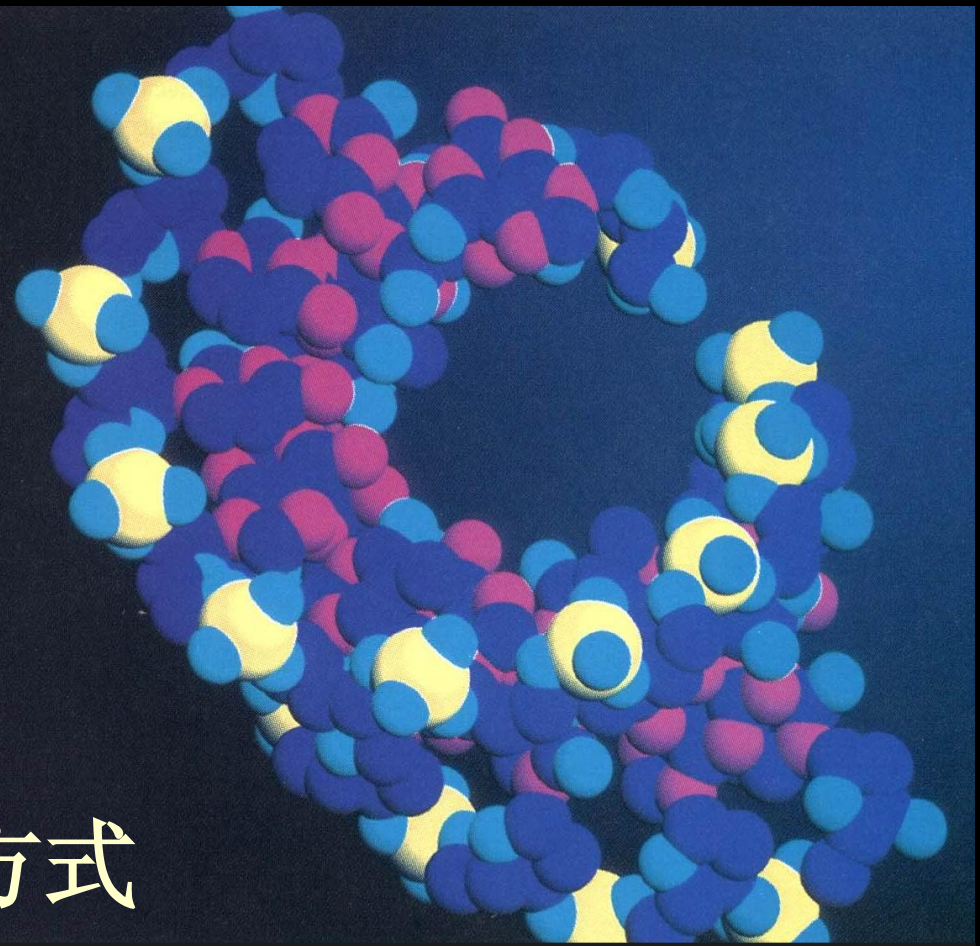
Okazaki 片段在某种意义上为 **dUMP** 片段

先导链按 **dUMP** 片段连续复制

后随链按 **Okazaki** 片段不连续复制

(Prok. 1-2kb, Euk. 0.1-0.2kb)

Source:Ken Eward/SS/Photo Researchers,inc



3.3. DNA复制的方式

(DNA replication model)

● Rifampicin 是E.coli RNA polymerase 的抑制剂

primer

S.S. DNA virus



E.coli

[Rif^S]

~~DNA聚合酶不能发动子链DNA的复制起始!~~

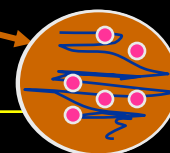
无M13 RF

E.coli

[Rif^S]

+ M13

Rifampin



有M13 RF

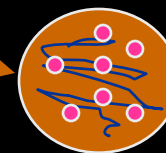
E.coli

[Rif^R]

Rifampin



M13



有M13 RF

Conclusion

- M13 RF的形成需要 RNA polymerase 发动合成一段 RNA 分子作为引物
- RF启动后, RNA引物已经形成, Rifampin 的抑制无效

The first evidence supporting RNA priming

DNase cannot completely destroy Okazaki fragments

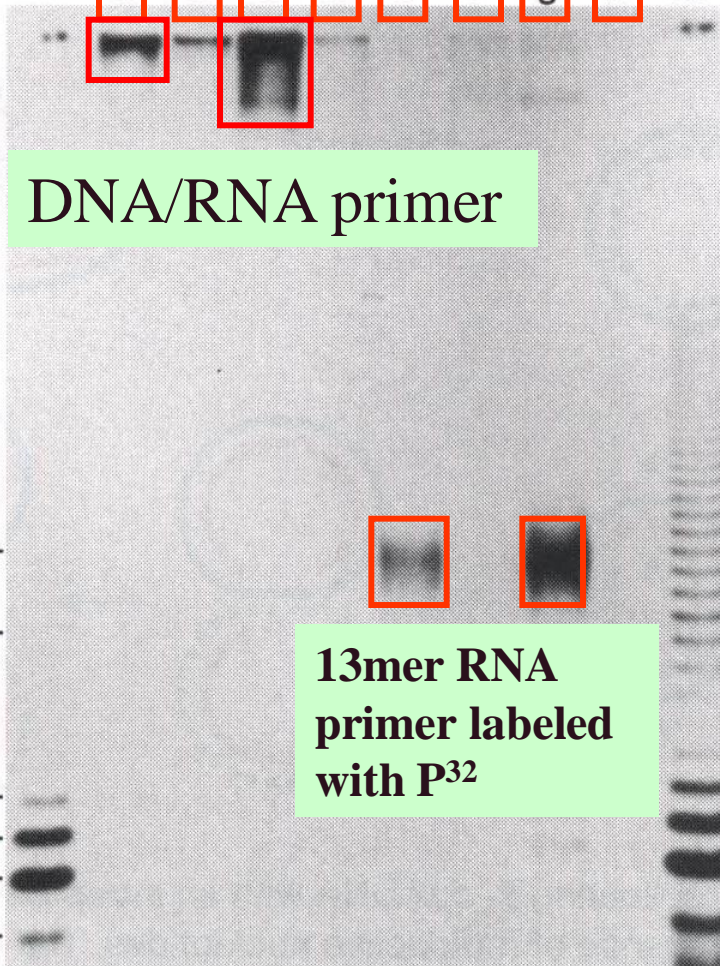
Before

After

DNase digestion

DNase digestion

M a b c d e f g h M



(Source: Tuneko Okazaki *J. Mol. Biol.* 184 (1985) p. 49)

labeled the intact primers on the Okazaki fragments with [32p]GTP.
destroyed DNA with DNase, leaving only the labeled primers.

genotype

RNaseH DNase

a and e;	-	+
b and f ;	+	-
c and g;	-	-
d and h;	+	+

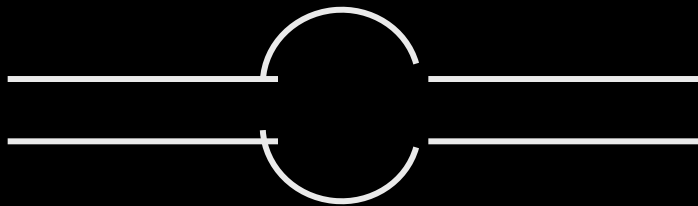
- 新起始方式 (de novo initiation)

或 复制叉式 (replication fork)

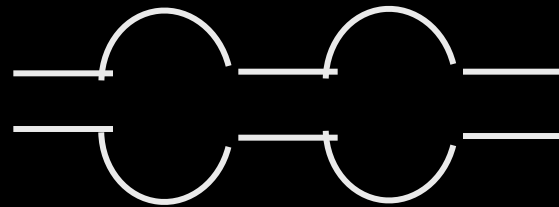
starting point → RNA primer → transcription activation

→ leading strand → fork → lagging strand

(Cairns model , θ form, theta form)

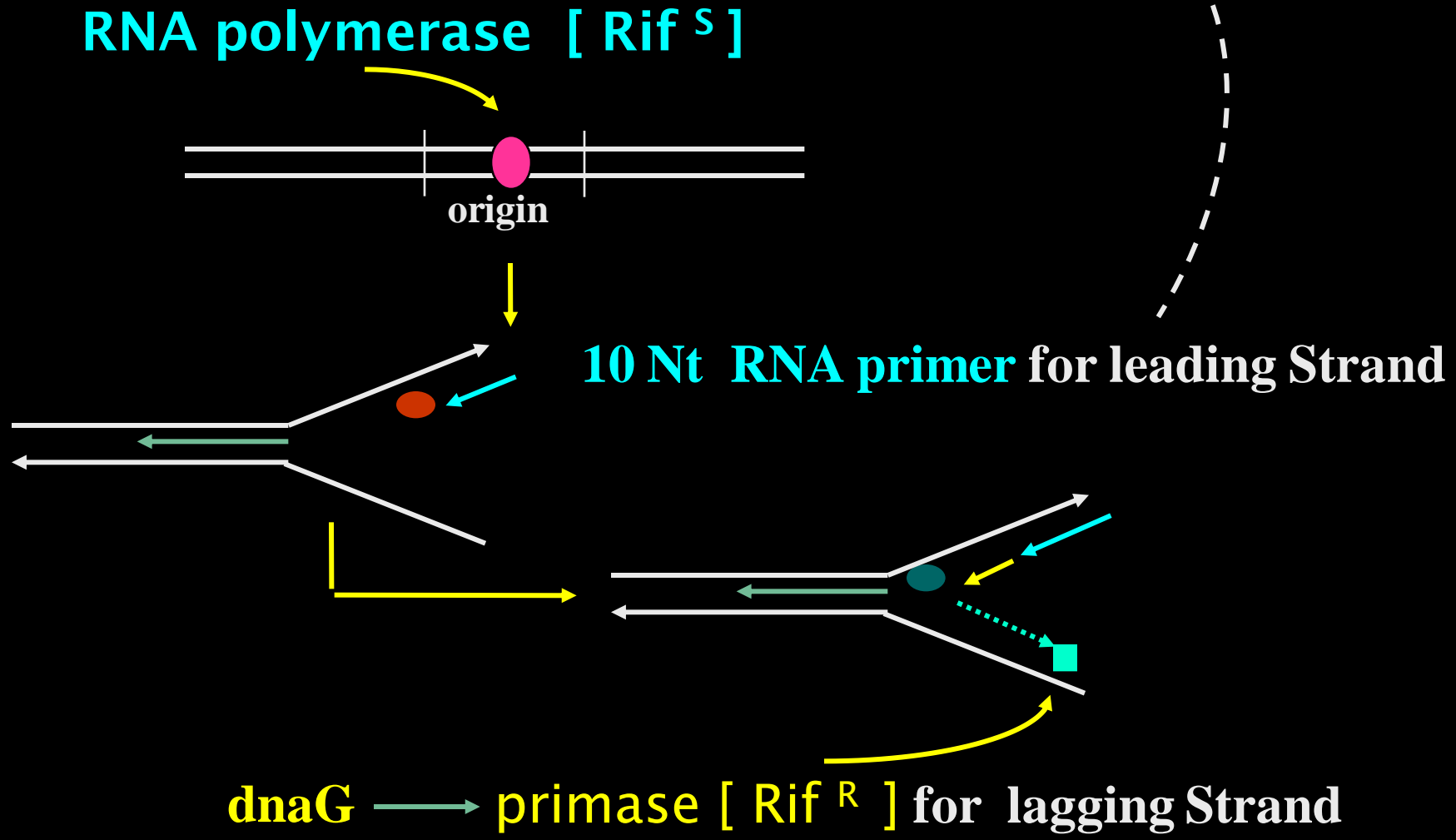


Eukaryote(500-5000bp/min)

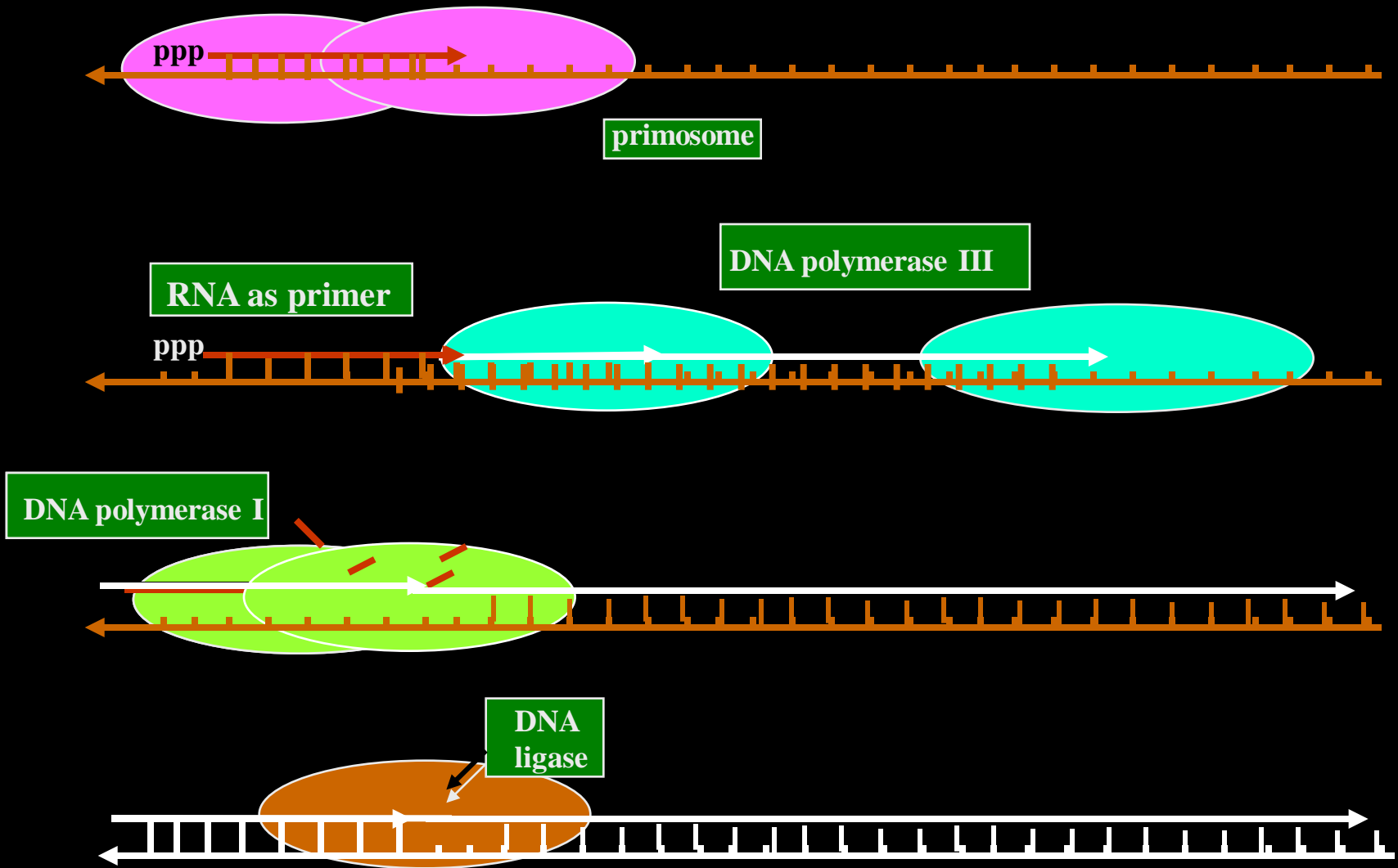


multiple replicon

● DNA复制的转录激活 (transcriptional activation)



Synthesis of progeny strand in lagging DNA



Conclusion

- RNAPol (RNA polymerase) [Rif^S]
完成对先导链引物的合成
实现DNA复制的转录激活起始
- dnaG (primase) [Rif^R]
完成对后随链引物的合成
较先导链的启动落后一个Okazaki片断
- 完成10 ± NtRNA引物合成后.
DNAPolIII进行DNA链的延伸
- DNAPol I 对RNA引物切除并聚合填补
- 连接酶 (ligase) 将 Okazaki 片段， dUMP 片段连接

- 共价延伸方式
(covalence elongation)
或滚环方式

(rolling circle)

D.S. DNA

→ Nick

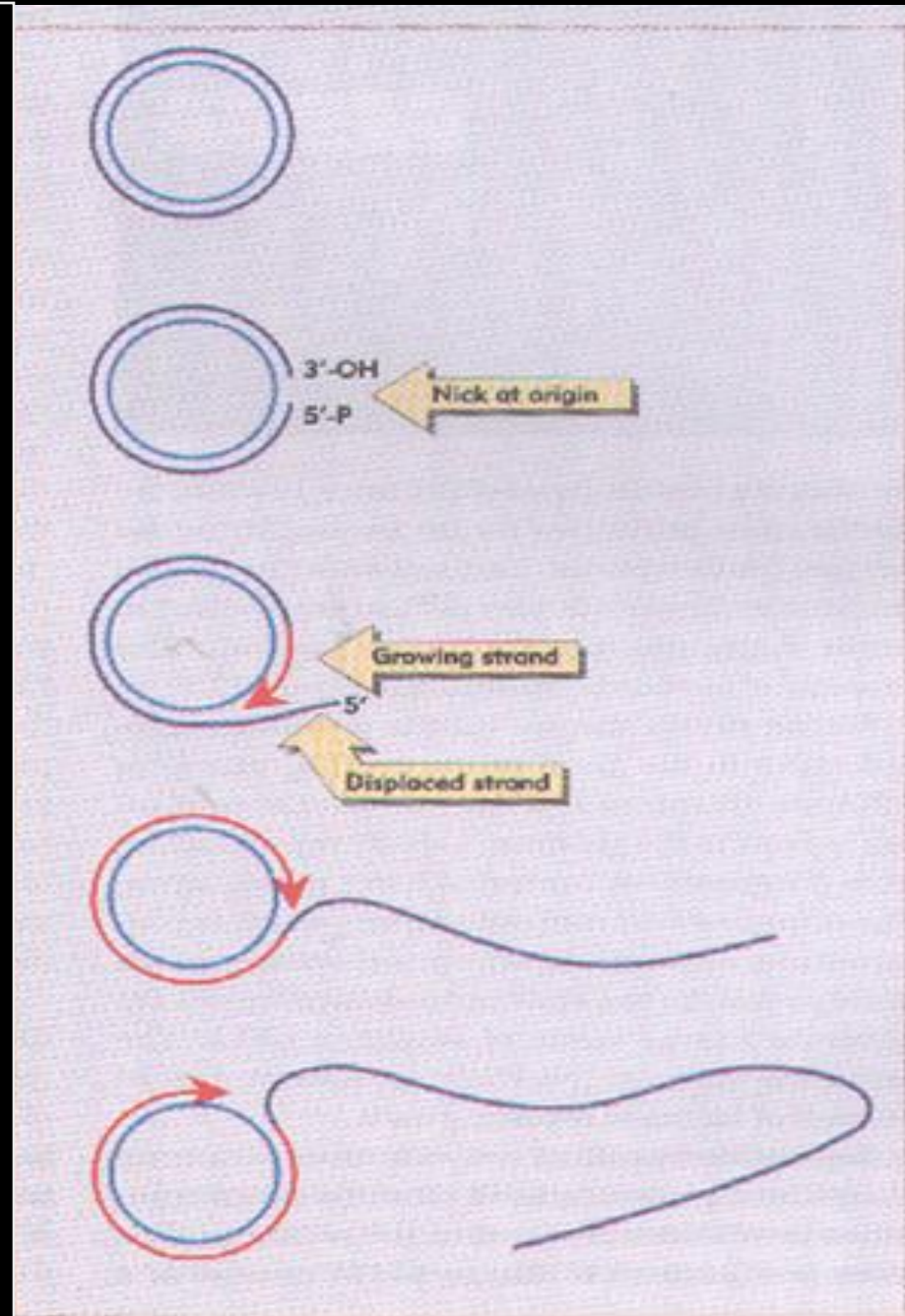
→ leading Strand

→ Elongation

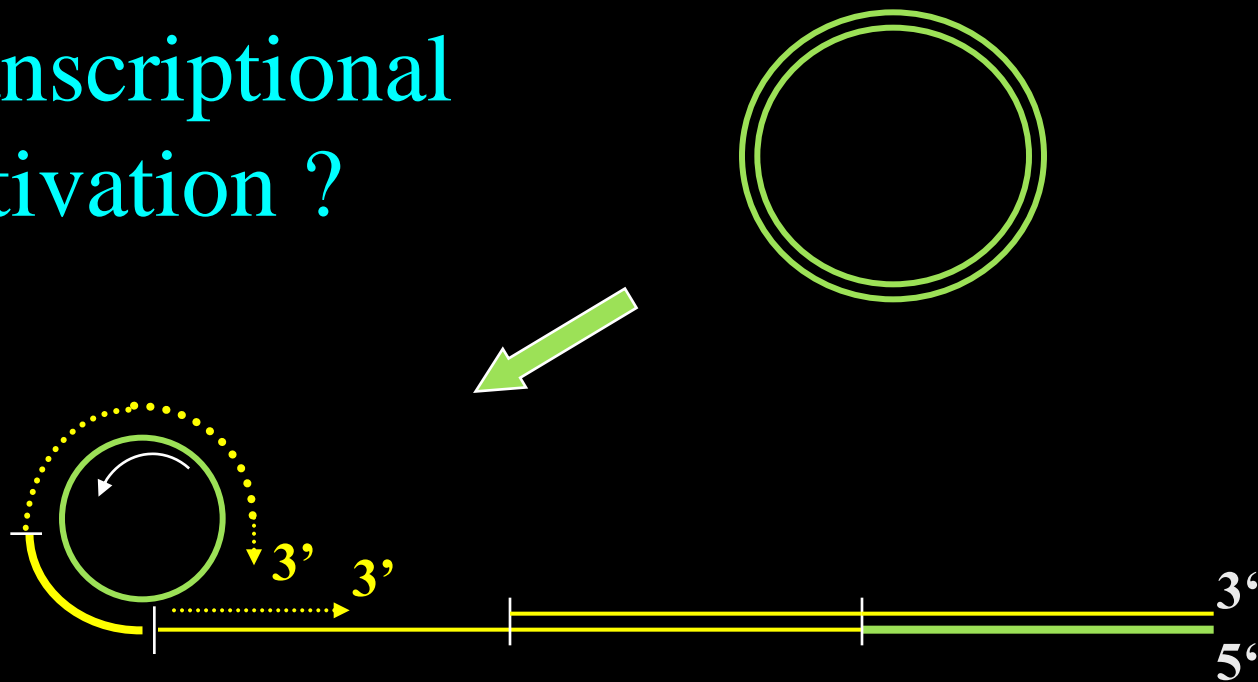
→ rolling

→ Lagging Strand

(δ form, delta form)



transcriptional
activation ?



共价延伸方式

● 置換式

(Displacement form)

D.S. DNA

→ S.S. DNA as template

→ New S.S. DNA

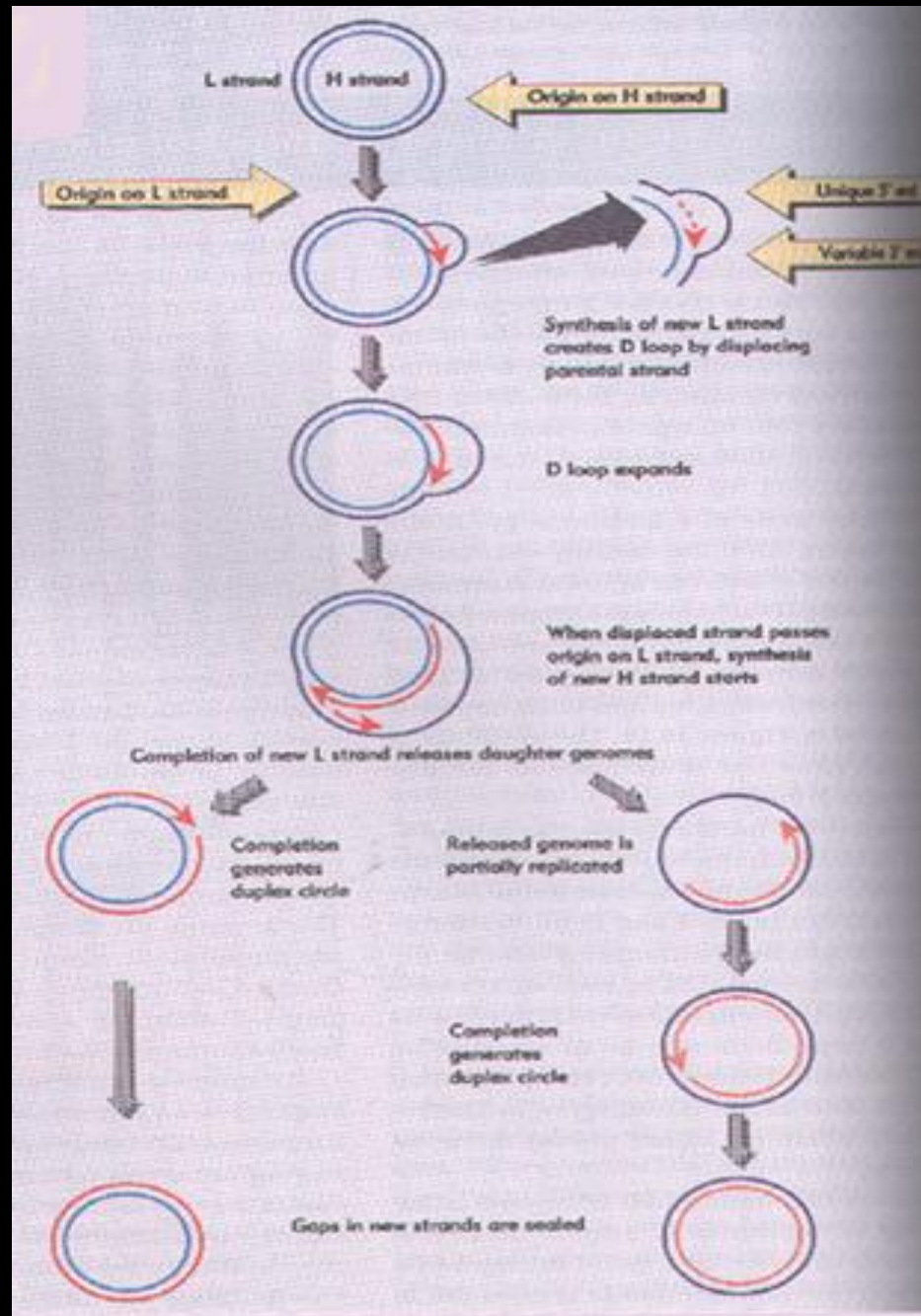
→ Displacement

→ D-Loop

In mitochondrial DNA

also in chloroplast

DNA



复制叉两侧DNA双螺旋的解旋

E.coli

$C / \text{genome} = 4.2 \times 10^6 \text{ bp}$

30-40' / 每次复制历时

10 bp / 每圈螺旋

84000 - $10^5 \text{ bp} / \text{min}$ 解旋 / 分

(8400-10000 rpm ! 高速离心机)

能量?

复制叉两侧DNA双螺旋的解旋

DNA Helicase (DNA 解旋酶)

ATpase活性, 解除 1氢键 / 水解2ATP

DNA topoisomerase I DNA的单链瞬间断裂

(transient single-strand break)

缓解高速旋转, 引入正向超螺旋, 解除overwinding的应力

DNA topoisomerase II DNA的双链瞬间断裂

(transient double-strand break)

缓解高速解旋时, DNA双链的相互缠绕, 产生负超消除复制叉移动时产生的正超

进化中形成了灵活的多酶复合体

replisome

复

制

体

Top I , Top II

Helicase (rep protein)

Single Strand Binding protein (SSB)

Helix destabilizing protein (HDP)

DnaB protein

DnaC protein

for primosome

Primase (dnaG)

Ung-ase

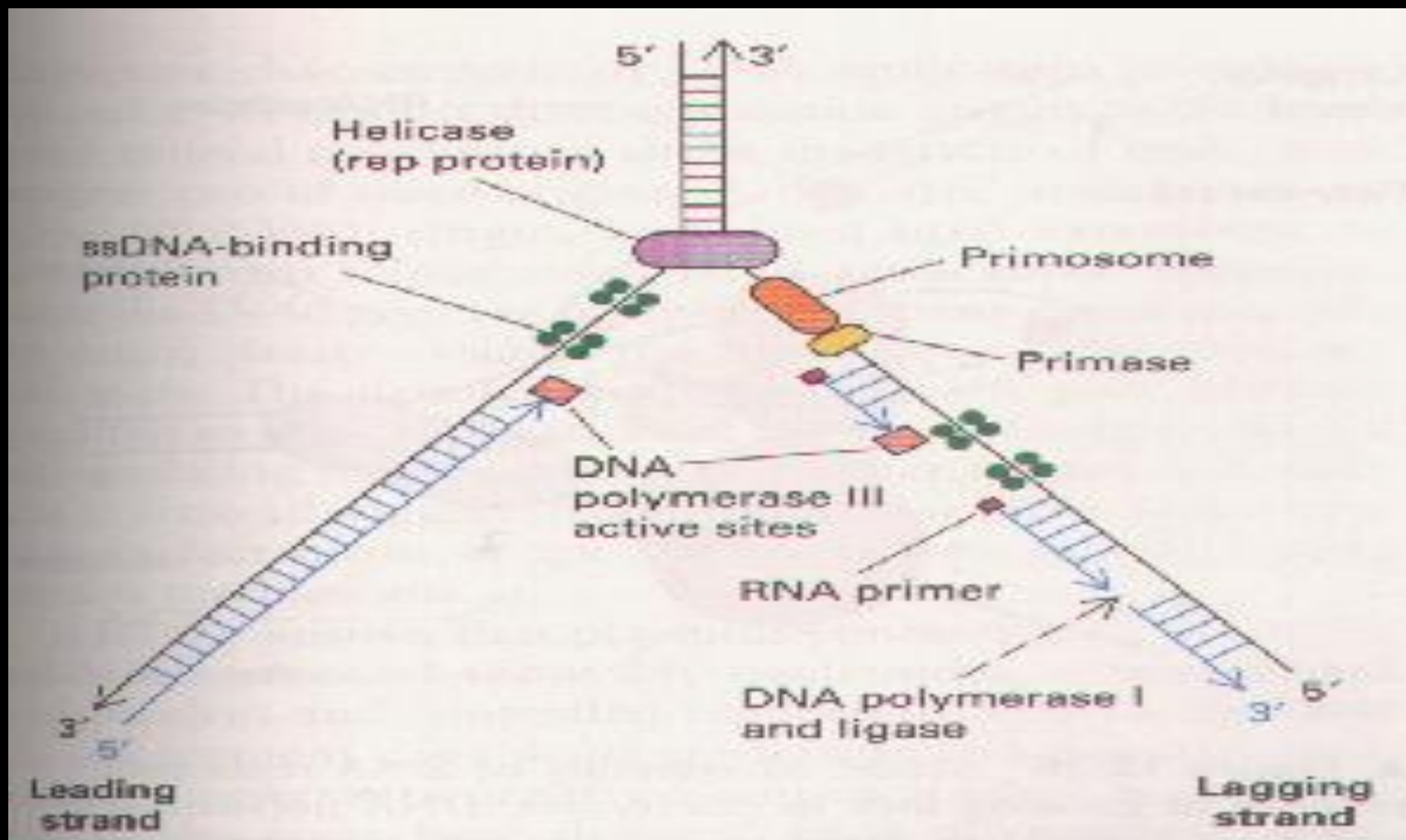
DNA Polymerase III

DNA Polymerase I

Ligase

进化中形成了

灵活的多酶复合体 —— replisome

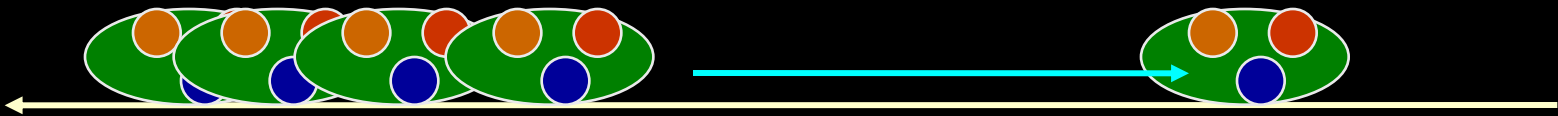


(来源: 不详)

位于复制叉处的多酶复合体

完成 lagging Strand DNA延伸时

- 必须快速从一个冈崎片段移到另一冈崎片段

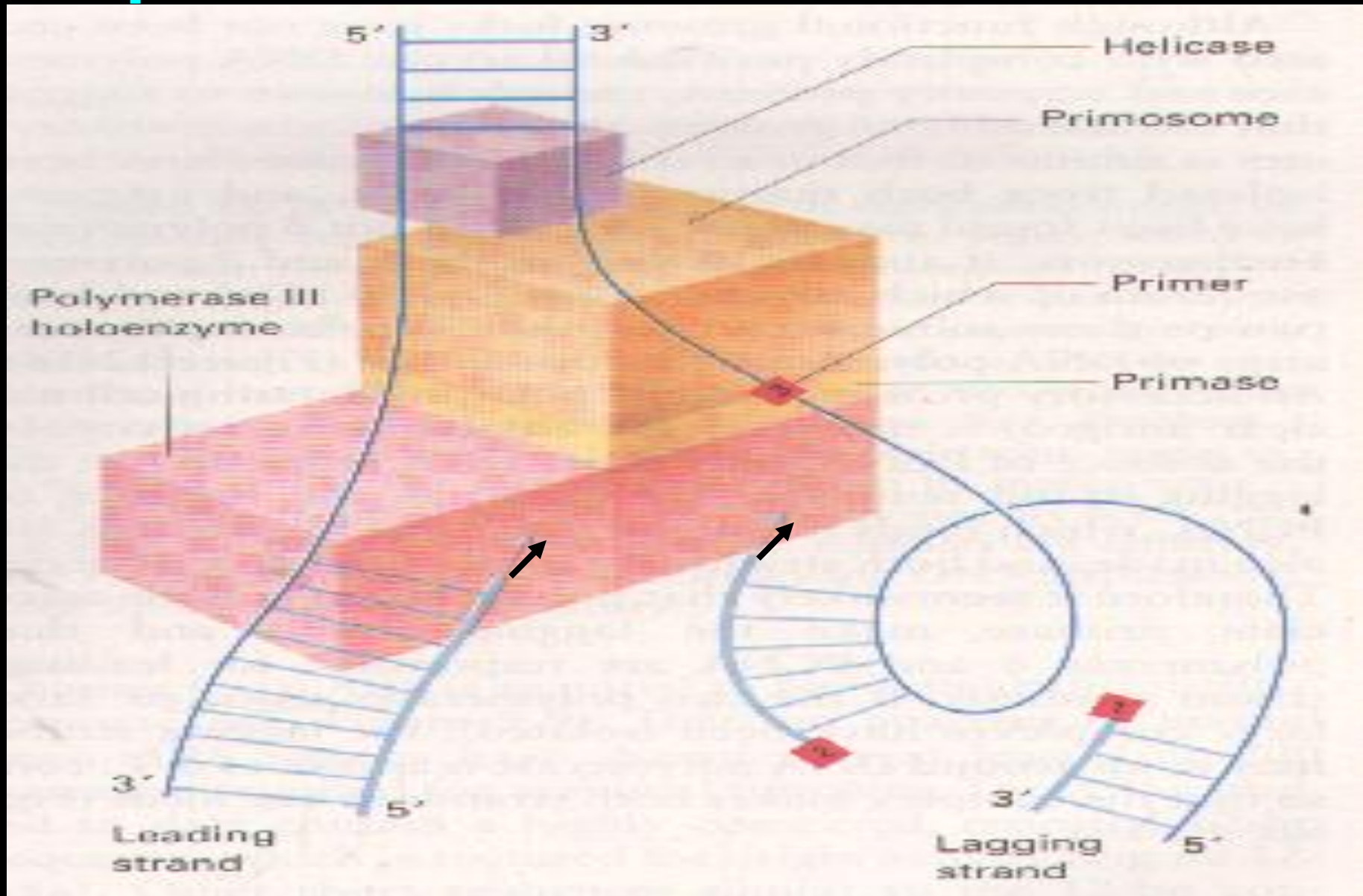


- In lagging Strand 多酶系统

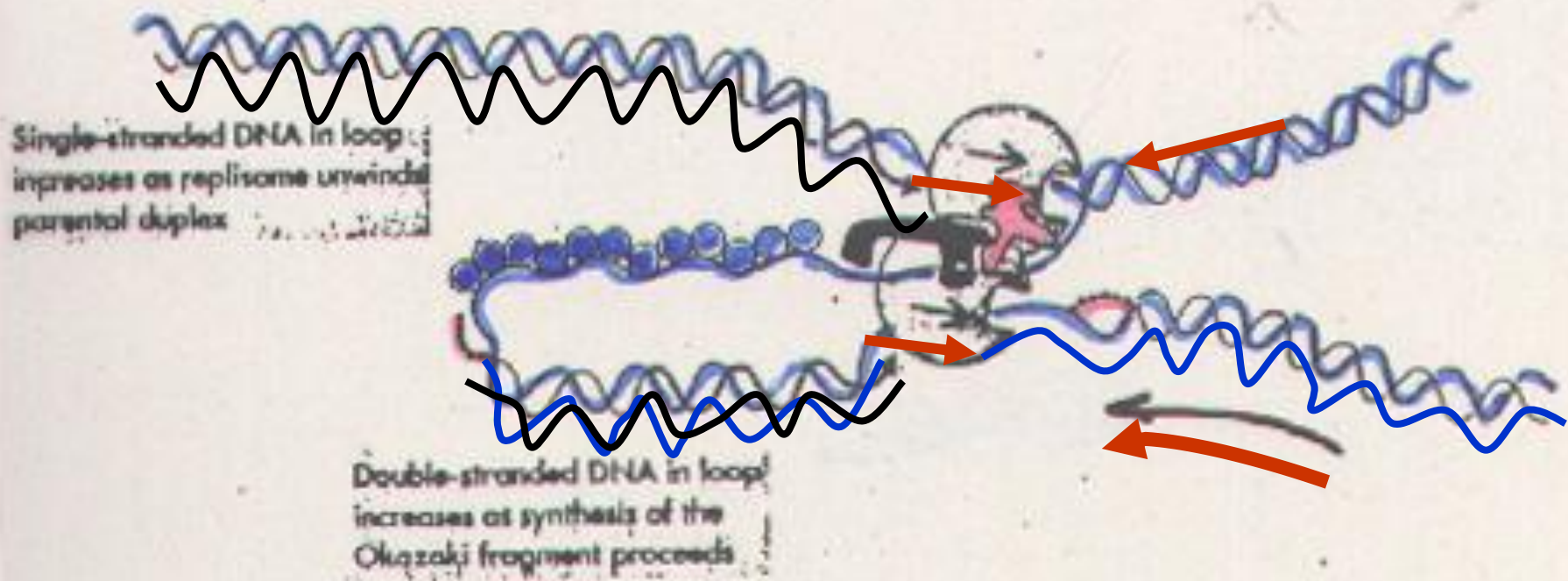
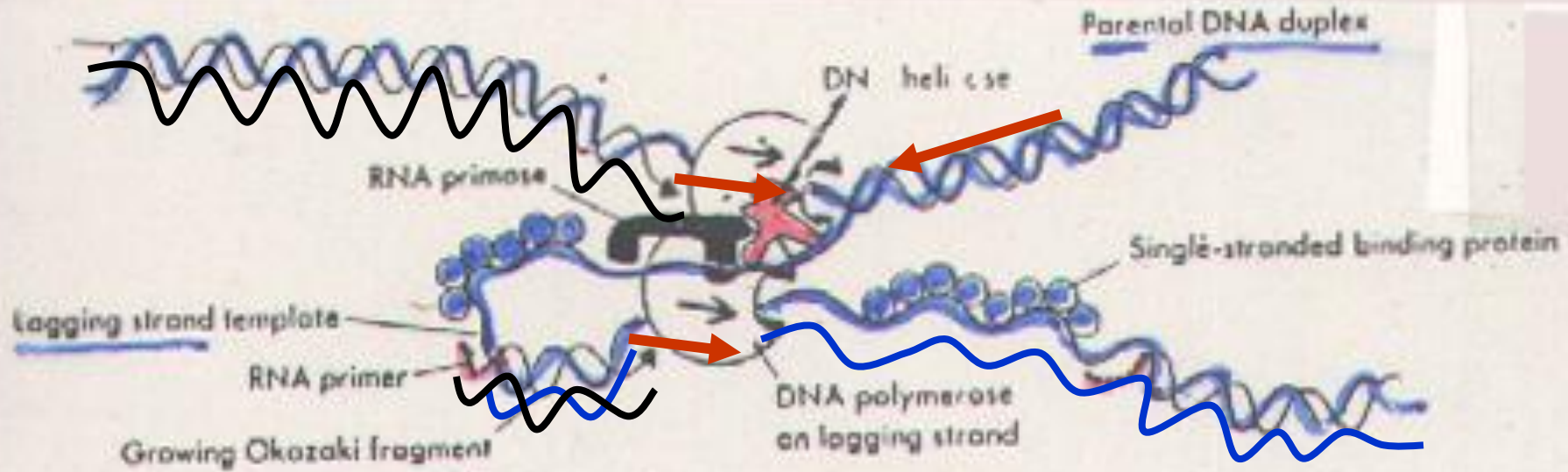
必须完成多次反复的启动与扩展

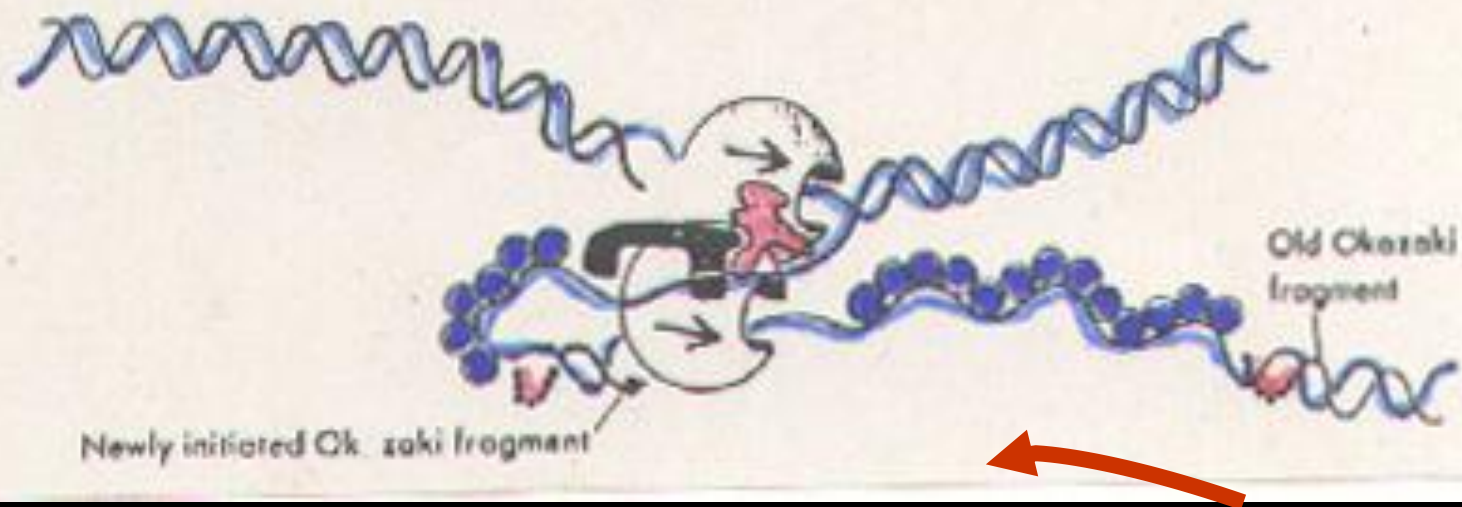
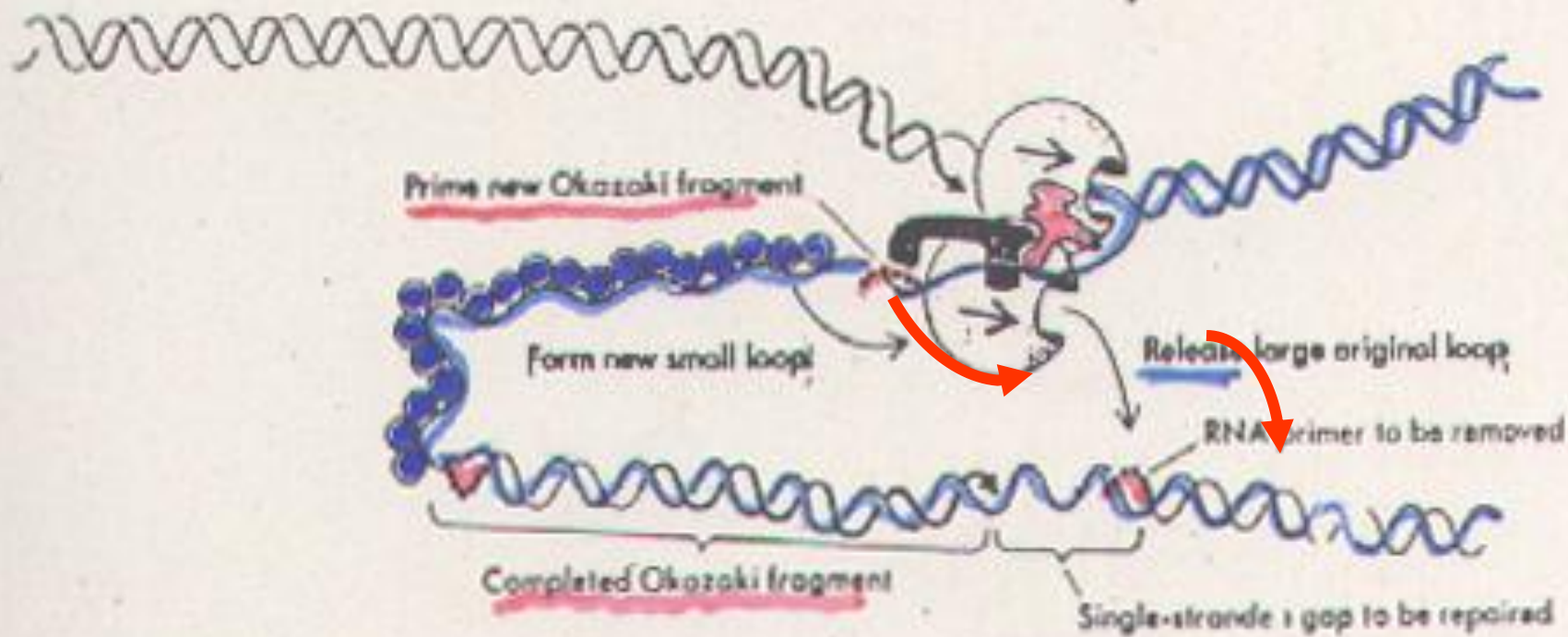
E.coli 启动2000—4000次的冈崎片段复制

Replisome model (后随链的回环模式)



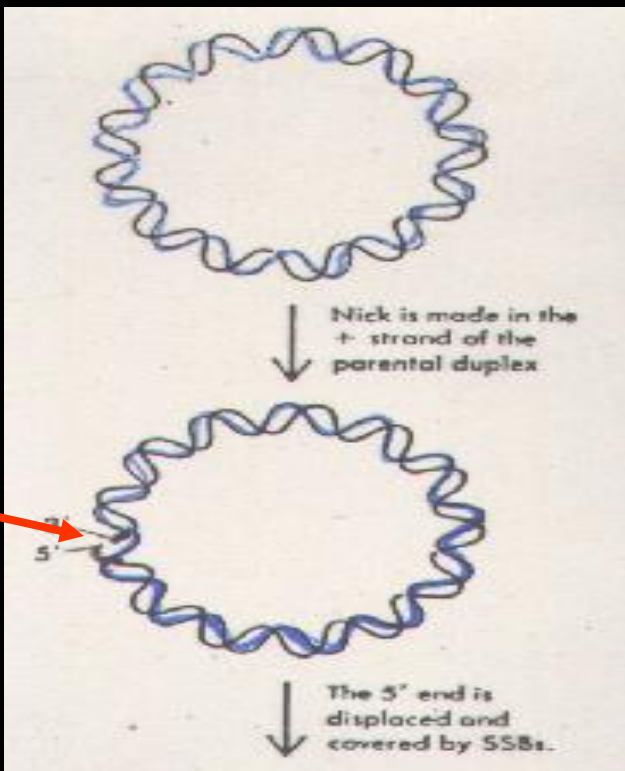
(来源：不详)



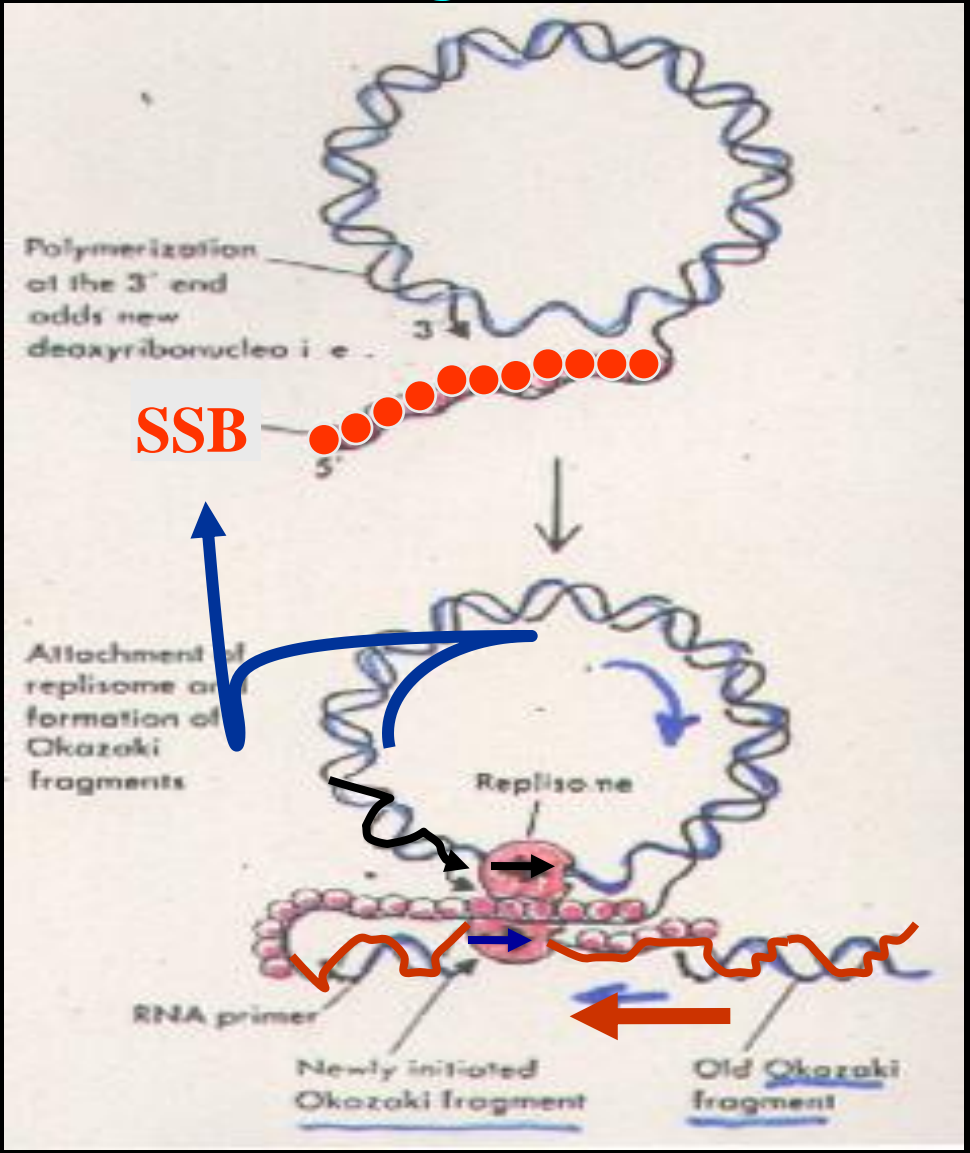


(来源：不详)

Replisome in covalence elongation

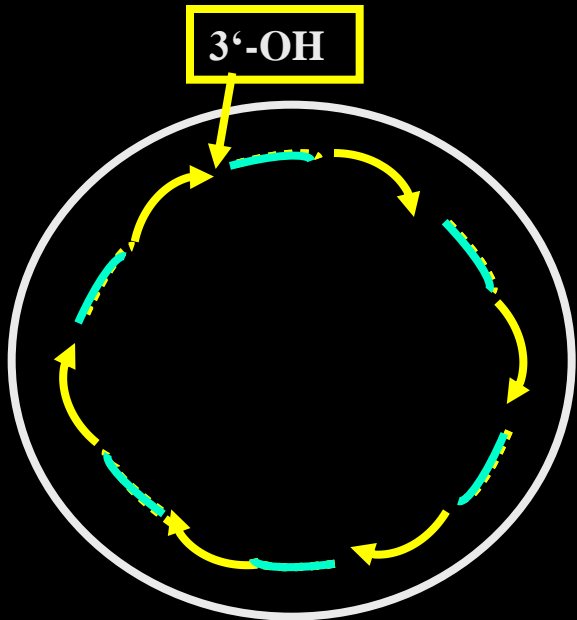


(来源: 不详)



(来源: 不详)

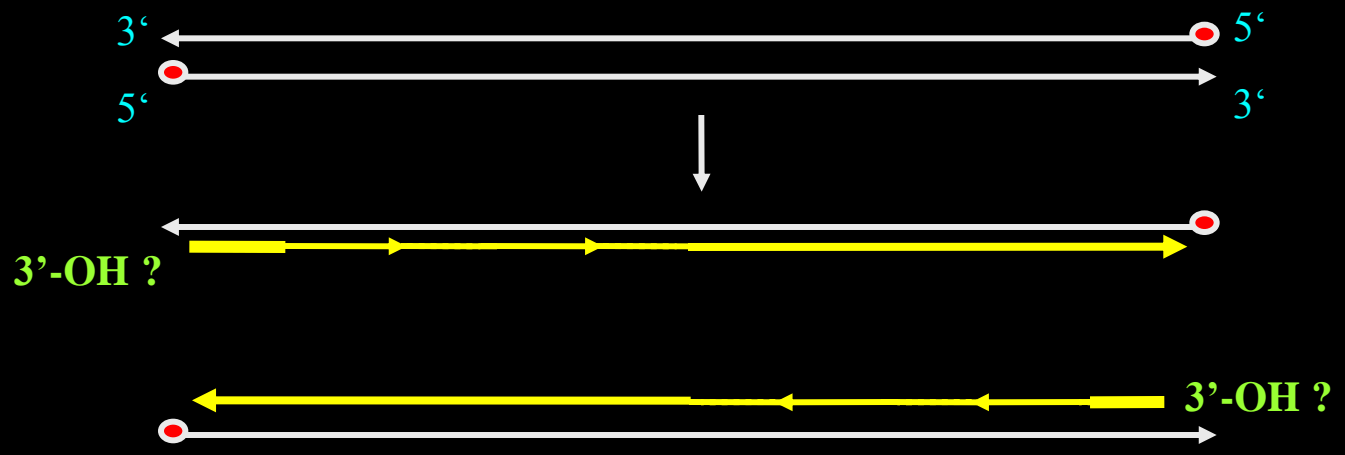
3.4. 线状 DNA 的复制 及避免 5' 末端短缩的模式 (5'-end shorten)



Lagging strand of circle DNA replication

But

Lagging strand of linear DNA replication



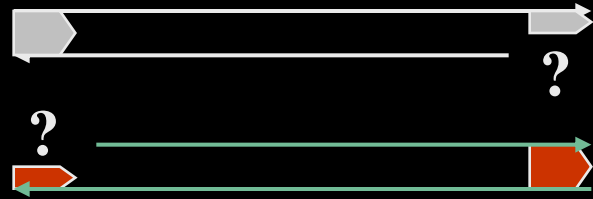
a) Watson J. D

T₇ phage

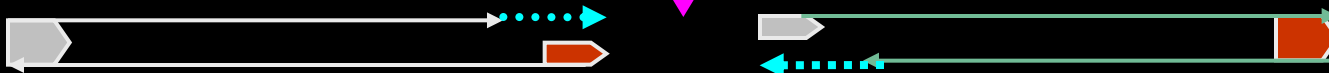
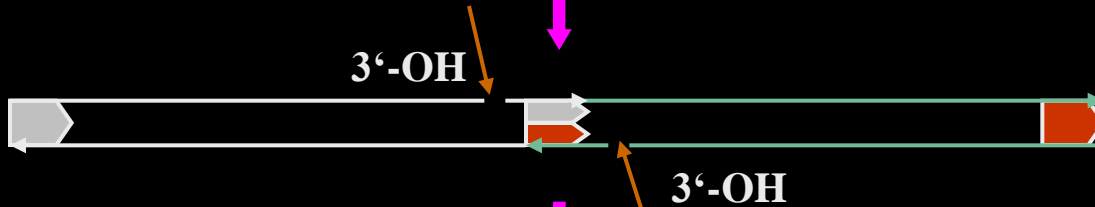
- **Direct repeat** in the end of linear DNA
(100 dNt terminal redundancy)
- **Concatemer** between two offspring DNA
after replication

ATCG
TAGC

ATCG
TAGC



concatemer



b) Replication of linear *Adenovirus-2* DNA



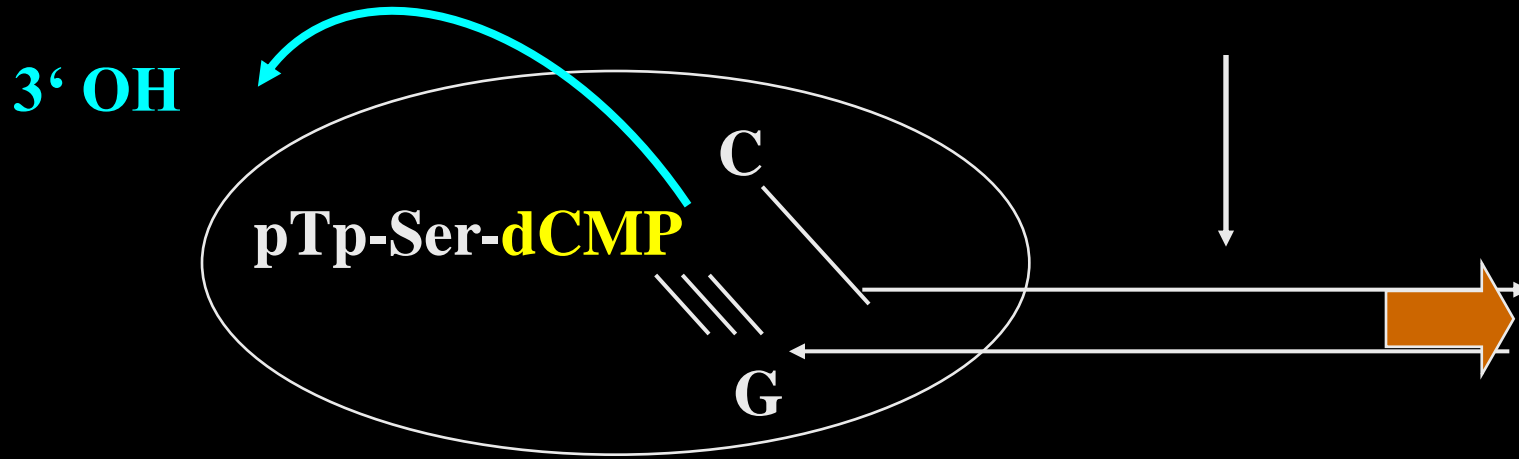
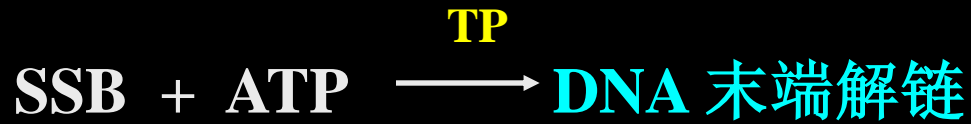
IR ; including 50 bp replication origin
rich AT & 1th C/G high conversation

pTP ; pre-Terminal protein 80 kd → TP 55 kd

SSB ; S.S. DNA binding protein 72 kd

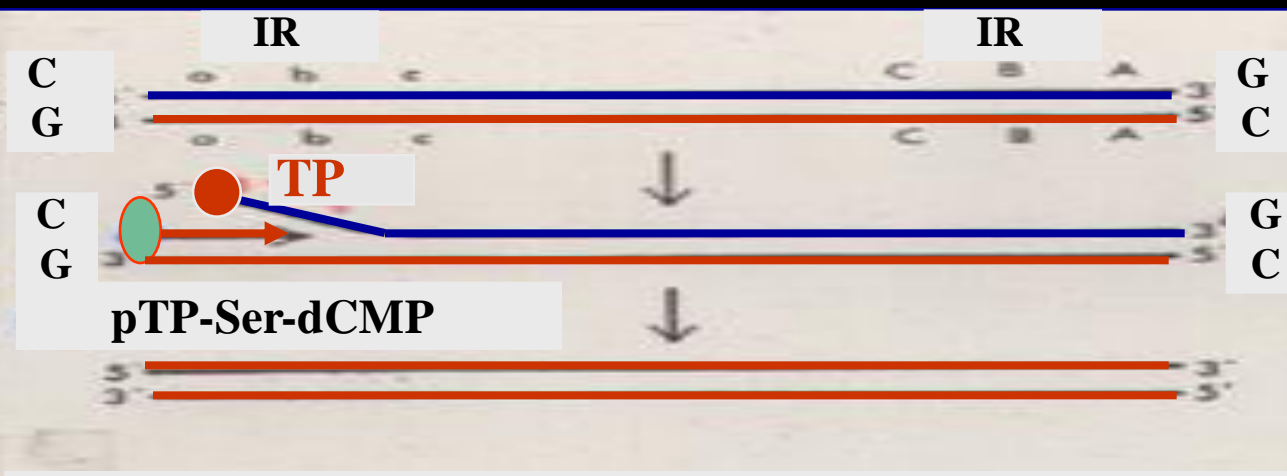
Ad2 DNA polymerase ; 140 kd

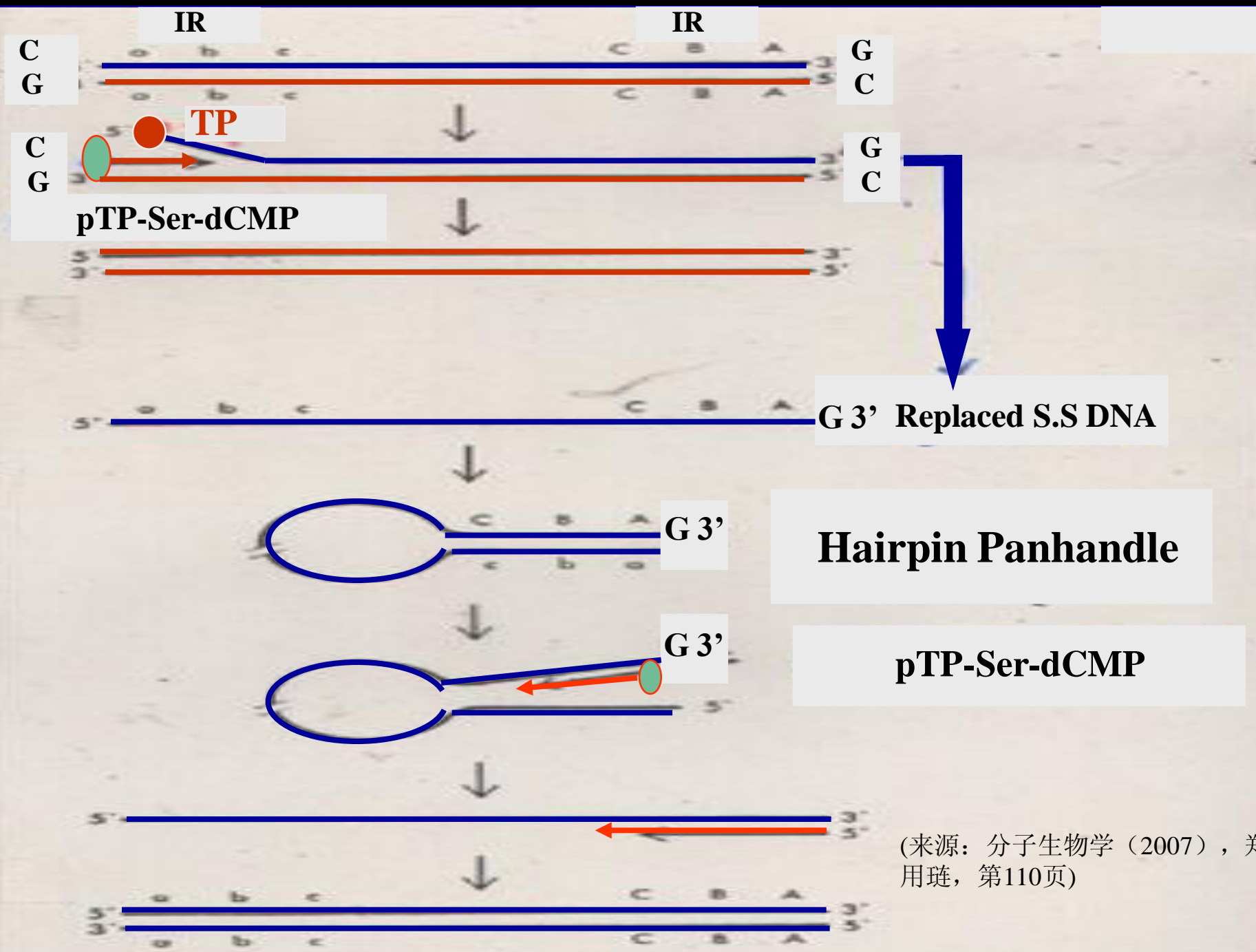
● 过程



复制起始复合体 \longrightarrow 引发复制 (不需引物)

避免5'-end shorten





G 3' Replaced S.S DNA

Hairpin Panhandle

pTP-Ser-dCMP

(来源: 分子生物学 (2007), 郑用琏, 第110页)

c) 真核生物染色体 DNA 末端补齐模式

● 端粒的发现

• *1938 Muller*

X-ray → *Drosophila*

末端极少发生缺失和倒位

推测染色体两端存在特殊结构，使染色体趋于稳定。

并定名为 **Telomere**

• *1938 B.McClintock*

顶端缺失染色体易于融合，而正常染色体不易连接。

推测染色体末端具有特殊端粒结构。

1970s 分子生物学发展 → 端粒研究获得突破



Elizabeth H. Blackburn

加利福尼亚
旧金山大学



Carol W. Greider

霍普金斯医学院



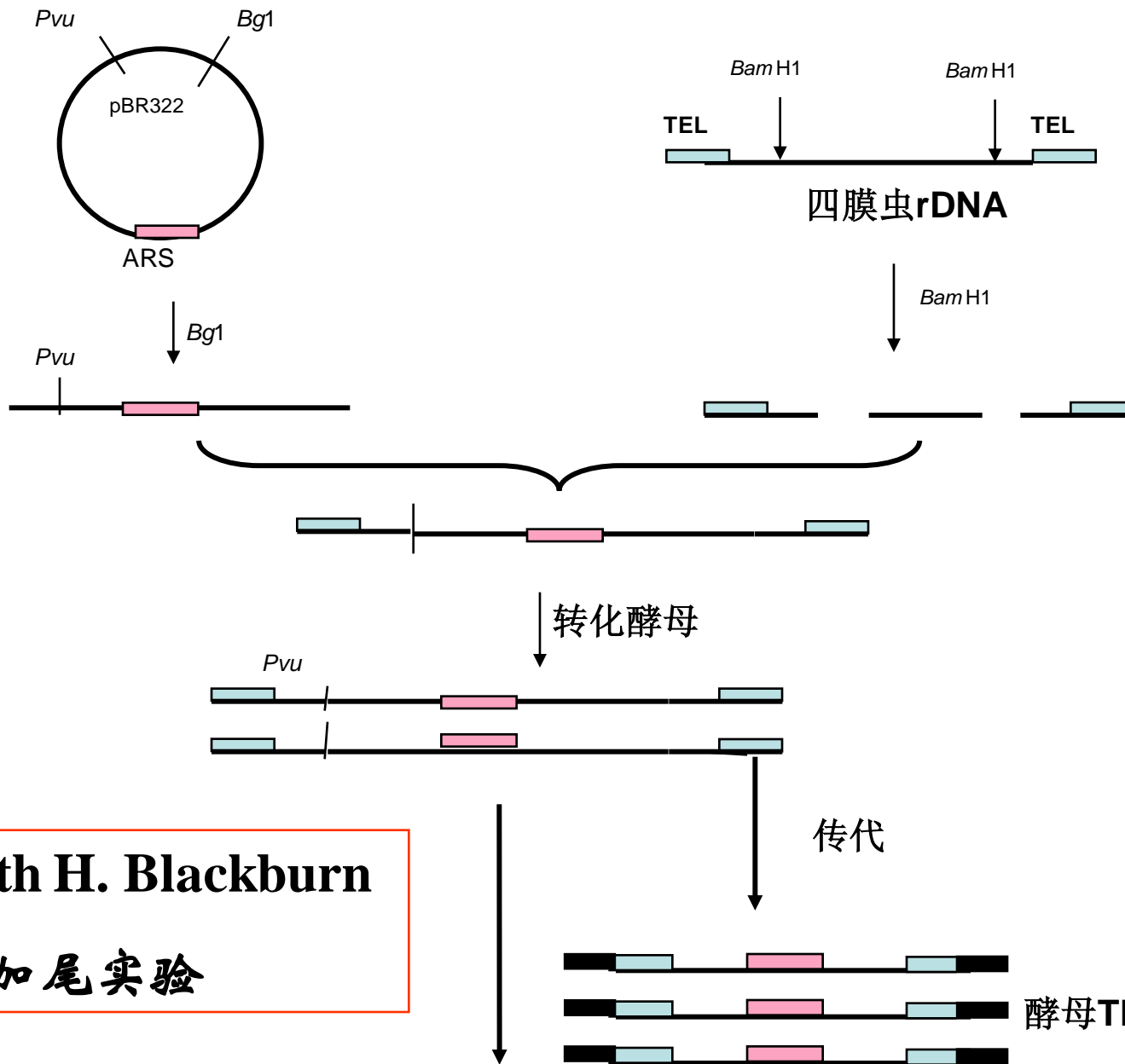
Jack W. Szostak

霍华德休斯
医学研究所



***for the discovery of how chromosomes are
protected by telomeres and the enzyme
telomerase***

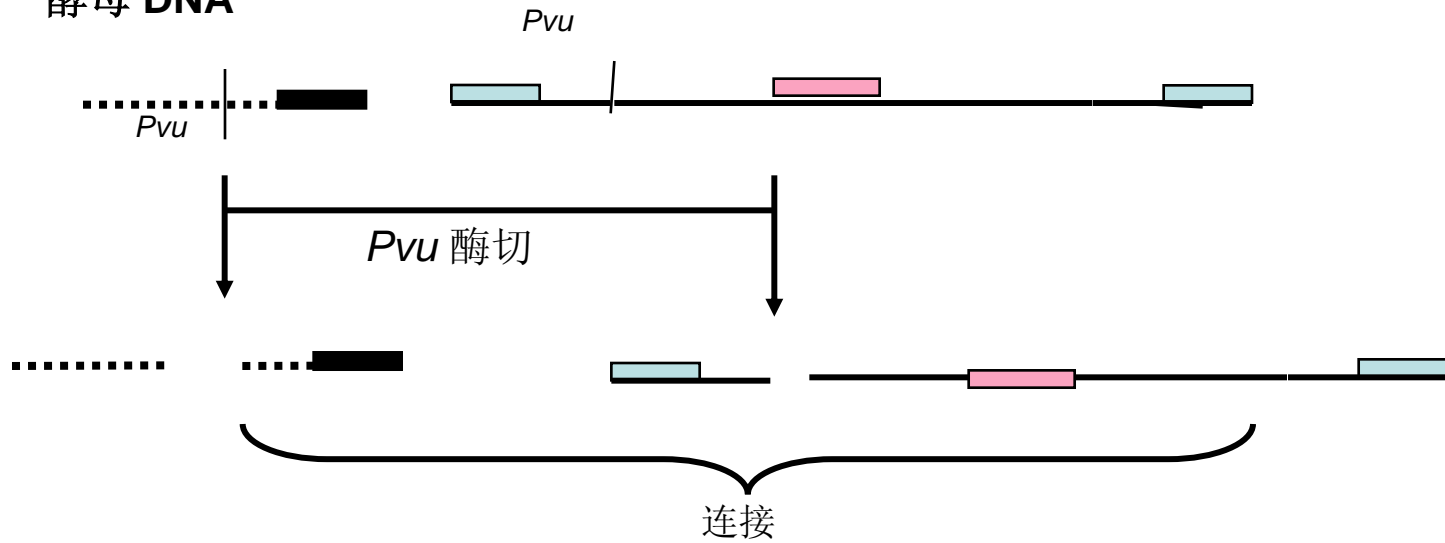
NP 2009生理学/医学奖



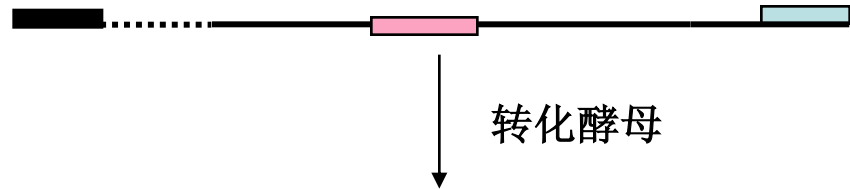
Elizabeth H. Blackburn
加尾实验

酵母TEL?!

酵母 DNA

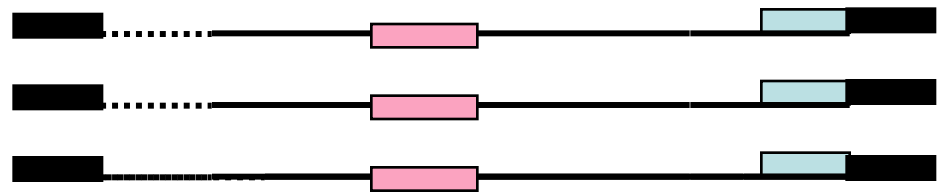


酵母 TEL



传代

酵母 TEL



酵母 TEL?!

端粒的模板链在何处?

端粒的模板链在何处？

加尾实验未能证明延伸端粒序列的模板从何而来？

端粒酶的发现为揭示端粒序列复制的模板及避免5'端短缩的机制奠定了重要的基础

- 端粒DNA (Telomere)

TTGGGG(T2G4)序列高度重复的末端



● 1985. **Carol W. Greider & Elizabeth H. Blackburn**

1986. **Gottchling**

尖毛虫 **telomere binding protein -1** 55kd
 telomere binding protein -2 26 kd } + 100 bp telomere

四膜虫 **telomerase**
游扑虫 **telomerase** } 200 – 500KDa

**RNA CAAAACCCC 链 + 具有逆转录酶活性的
 末端结合蛋白(TBP)**

1990 Yu和Blackburn 在体外加尾实验的反应体系中

加入这段RNA区域的反义序列GTTTTGGGGTTG



端粒酶的加尾功能
能受到明显抑制

证明端粒酶中CAACCCCAA序列是端粒重复序列 (GGGGTT) n 合成的模板

1990 Yu和Blackburn 在体外加尾实验的反应体系中

对CAACCCCAA序列进行诱变



转化四膜虫

后代的染色体端粒出
现了突变序列的端粒

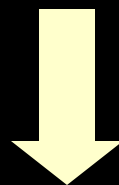
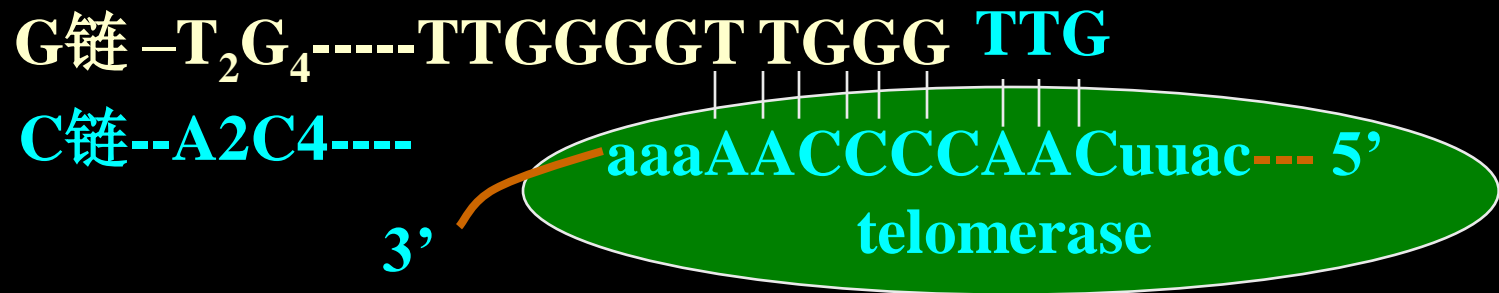
证明端粒酶中CAACCCCAA序列是端粒重复序列 (GGGGTT)_n合成的模板

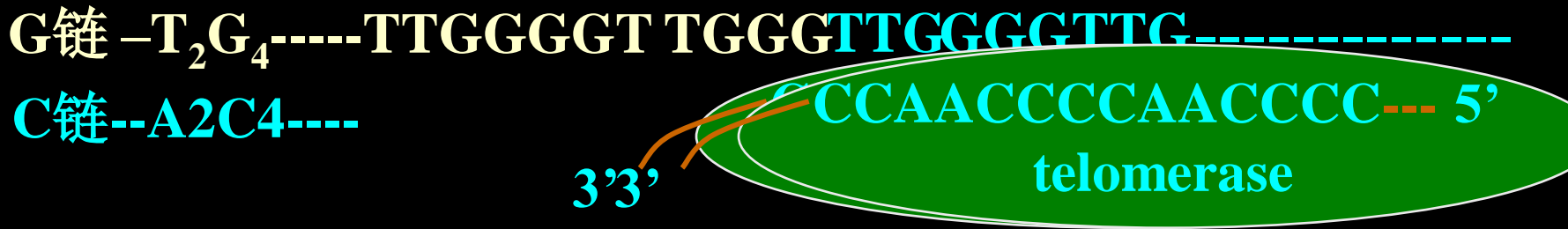
TBP is **Reverse transcriptase-like** **model**

RNA complement with 3' end of DNA

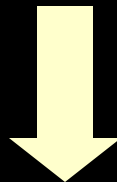
& as template for cDNA

Elongated T₂G₄ 3'-end as primer for 5'-end DNA synthesis





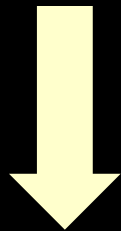
When G-rich strand is longer enough



G鏈 -T₂G₄-----TTGGGG TTGGGGTTGGGGTTGGGGTTGGGG
 C鏈--A₂C₄----- **DNA pol** CCAACCCCAATATSCCAACCC



or



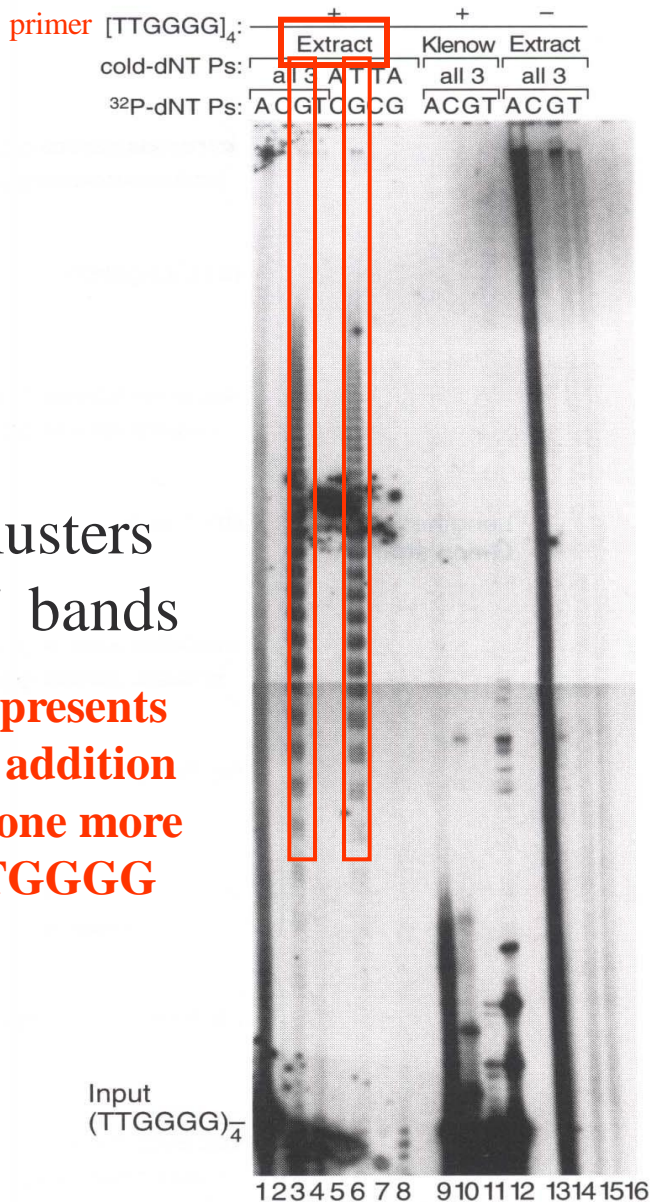
G鏈 -T₂G₄-----TTGGGG t t g g g g t t g g
 C鏈--A₂C₄----- AACCCCAA g g g g t t g g



G = G hoogsteen bond

Tetraplex helix

Identification of telomerase activity.



端粒酶活性的测定 Greider和Blackburn制备四膜虫无细胞提取物,并与人工合成的四重TTGGGG端粒重复序列寡聚物一起孵育90分钟,加入标记或未标记过的Nt,

1-4行: 每一行含有一种标记的dNt和三种其余的未标记dNt。第一行标记dATP, 第三行标记dGTP。

5-8行: 实验中含有一个标记的dNt和仅一种未标记的dNt, 结果: dGTP+dTTP能够被进入端粒。证明端粒链只含G和T。

9-12行: 仅DNA聚合酶, Klenow片段, 不能进行端粒延伸。

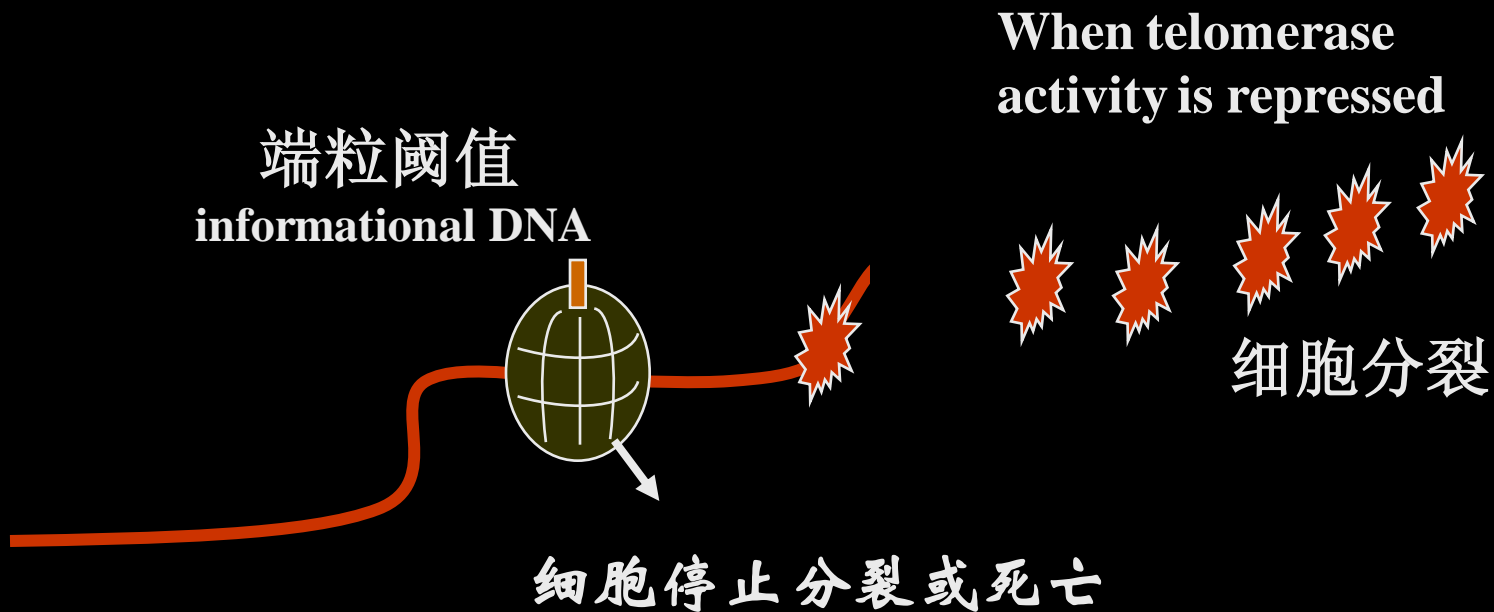
13-16行: 端粒酶的活性依赖于端粒酶的引物。

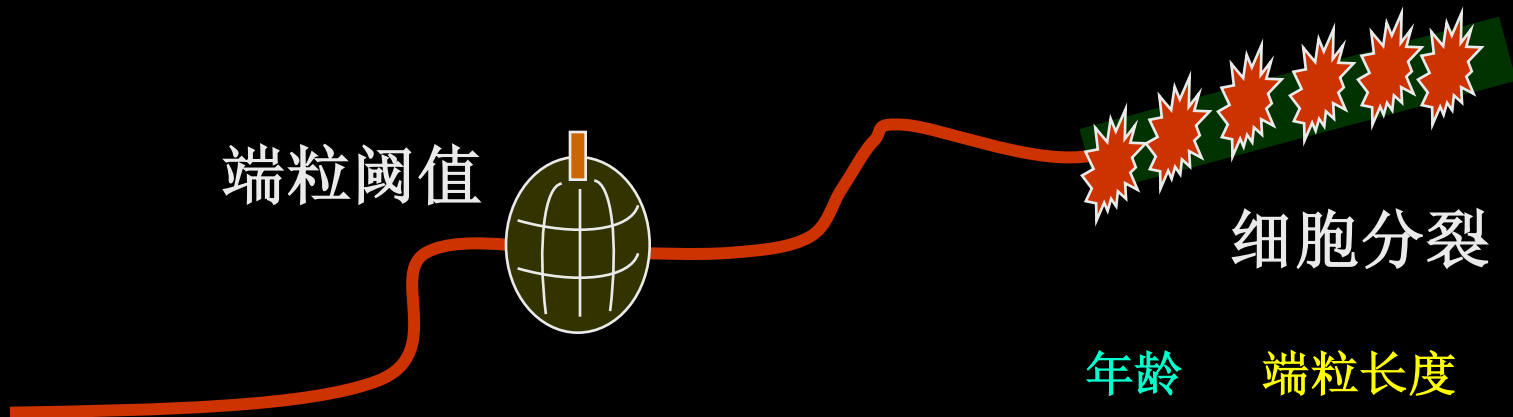
Source: Greider, C.W., and E.H. Blackburn, identification of a specific telomere terminal transferase activity in tetramere extracts. Cell 43(DEC Pt1 1985)

Clusters of bands

Represents an addition of one more TTGGGG

Telomerase activity is repressed in somatic cells of multicellular organisms resulting in a gradual shortening of the chromosome with each cell generation. As this shortening reaches informational DNA, the cells senesce and die.



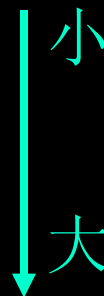


Harley (1989)

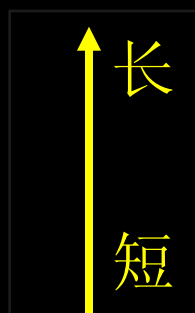
端粒的重复片段为探针检测 →

胎儿细胞株
 婴儿细胞株
 青年细胞株
 老年细胞株

年龄



端粒长度



端粒长短

端粒酶活

人体发育完成，端粒酶被抑制，

细胞分裂次数与端粒长短呈反比

早衰性侏儒症的端粒明显较正常人短

“多莉”的衰老

研究端粒（记时器）丢失的速率/年，预测人类的寿命

XX > XY why?

生殖细胞、癌细胞的繁殖（端粒酶的激活！细胞得以永生！）

百年人参 的鉴定？



较老的胚, 30 kb

未成熟胚, 80 kb

成熟的穗状花序, 20 kb

幼嫩的穗状花序, 45 kb

叶, 23 kb

随着组织、细胞的老化
染色体端粒的长度变短

(来源: 分子生物学 (2007), 郑用琏, 第116页)

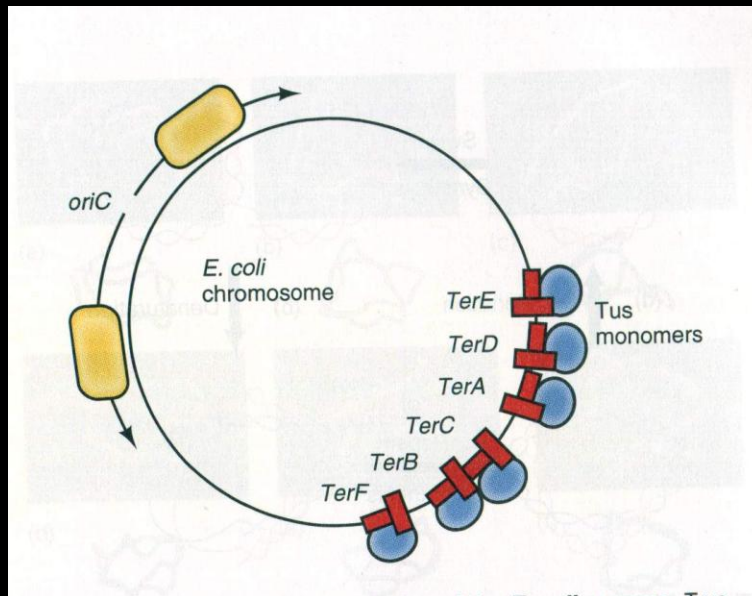
The termination of DNA replication

E. coli (单起点双方向)

两复制叉的相遇处具有多个终止位点(不是复制叉简单相遇)

terE, D, A **terF, C, B** (22bp保守区)

Terminus Utilization Substance (TUS 36kd)

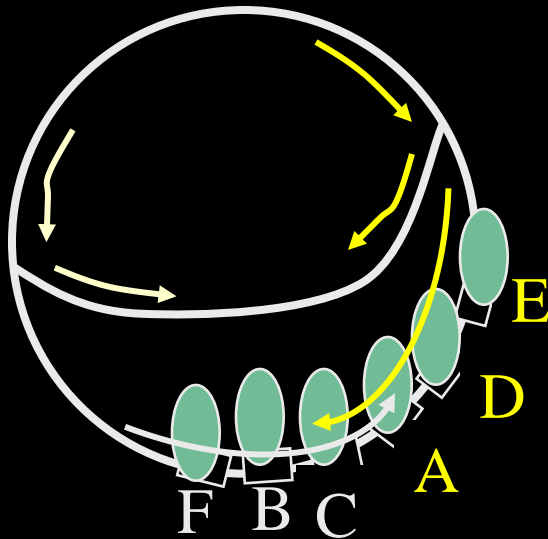


} Ter-TUS复合体

(source:Adapted from Baker,T.A,Replication arrest.Cell 80:521,1955.)

terE, D, A 仅对反时针方向的复制叉具有终止效应

terF, B, C 仅对顺时针方向的复制叉具有终止效应



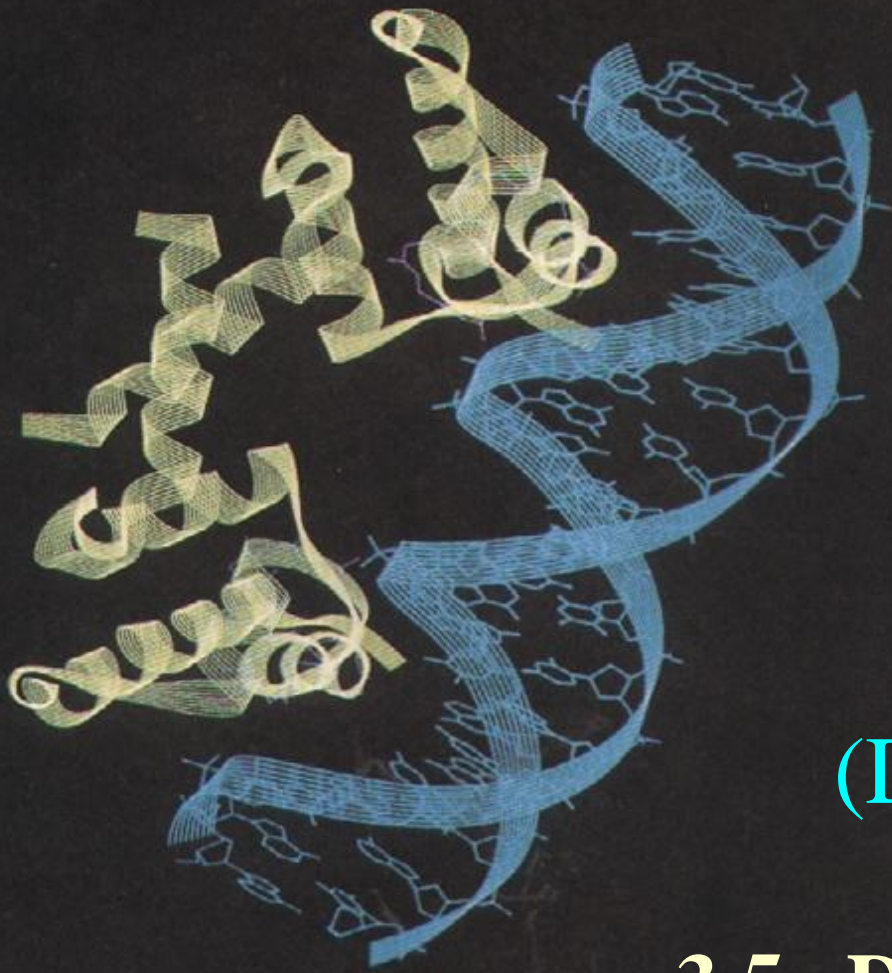
Ter-TUS复合体 → (Rep. fork trap)

终止DnaB(螺旋酶)

防止DNA过度复制

避免出现DNA多聚体，高拷贝

氨基酸饥饿状态，DNA复制起始失控



第3章 DNA复制

(DNA Replication)

3.5. DNA复制相关调控与修饰

(来源：不详)

3.5.1 DNA的复制基因 与酶类体系

a) 原核生物的复制基因与酶类

Initiate genes

dna B	prepriming	300kd	~20 copies/cell hexamer
dna C	acts with dnaB	25kd	
RNApol	primase for leading S.		
dna G	primase for lagging S. (Okazaki fragment)	60kd	~ 25 monomer
SSB	Binding with S.S.DNA Prevents from anneal	74kd	~300 monomer

Elongate genes

dnaE	DNA polymerase III(α)	140kd	~20
dnaZ	DNA polymerase III(β)	52kd	~20
polA	DNA polymerase I	109kd	~400
polB	DNA polymerase II	90-120kd	~100

Topoisomerase

rep Relaxed protein (Helicase)

D.S. DNA \rightarrow S.S. DNA

ATPase 1H-bond / 2 ATP

HDP Helix De-stabilizing Protein

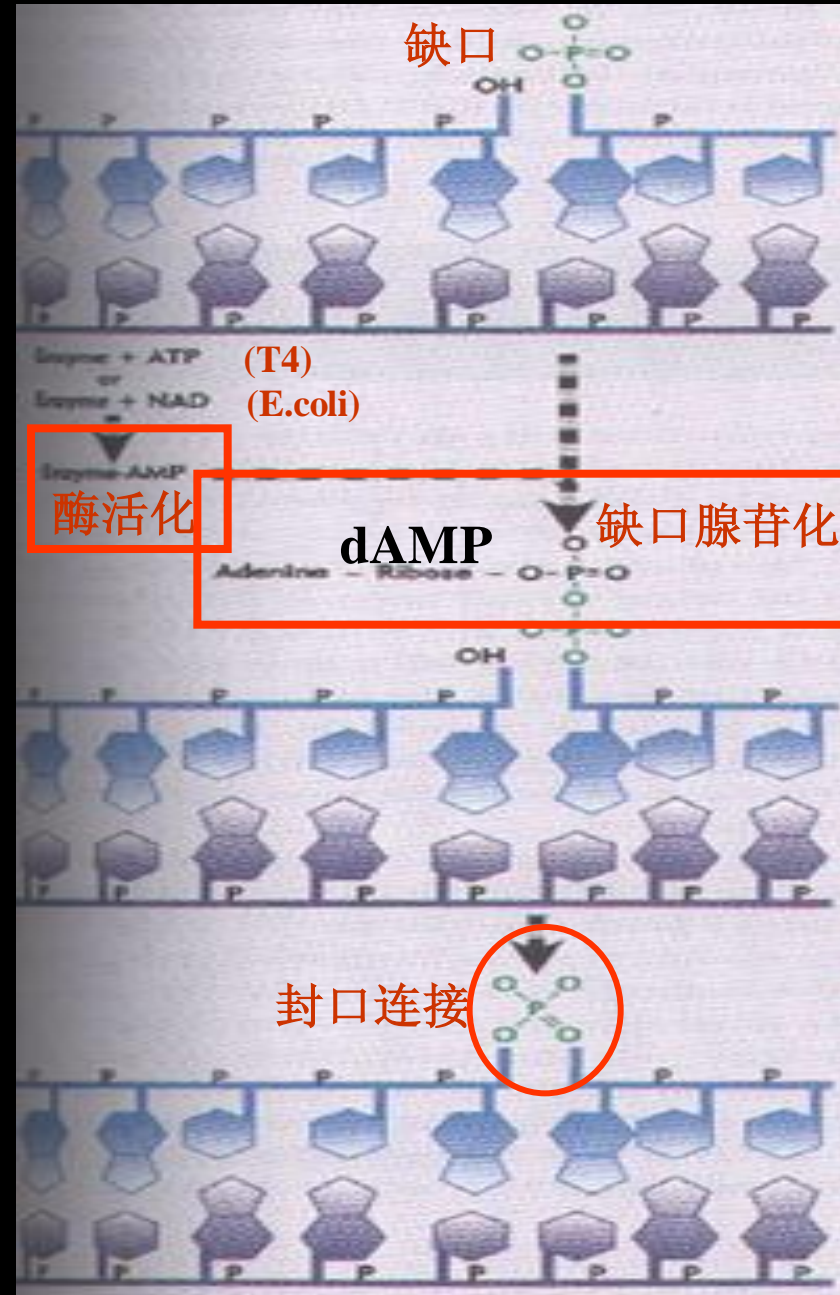
No ATPase activity

Binding S. S. DNA

decrease T_m

Prevents from anneal

lig Ligase



(来源: 不详)

b) DNA聚合酶 (DNA polymerase)

Prokaryote

I	II	III
<ul style="list-style-type: none"> • PolA 	<ul style="list-style-type: none"> polB 	<ul style="list-style-type: none"> dnaE dnaZ
<ul style="list-style-type: none"> • 109kd 	<ul style="list-style-type: none"> 120kd 	<ul style="list-style-type: none"> >250kd
<p>(75, 36) <i>Kornberg E</i></p>		<div style="border: 1px solid black; padding: 5px; display: inline-block;"> $\alpha\alpha + \text{EF-II}$ </div>
<p style="text-align: center;">↓</p>		<p style="text-align: center;">+</p>
<p><i>klenow</i> fragment</p>		<p>$\beta\beta$</p>
<p>monomer</p>		<p>hetero-multimer</p>
<ul style="list-style-type: none"> • 5' → 3' elongation 	$(\text{dNMP})_n + x\text{dNTP} \rightarrow (\text{dNMP})_{n+x} + x\text{ppi}$	$(\text{dNMP})_n + x\text{dNTP} \rightarrow (\text{dNMP})_{n+x} + x\text{ppi}$
<p>10 dNt/sec</p>	<p>1/10 of pol I</p>	<p>500dNt/sec ★</p>

I

II**III**

- **3' → 5' editing**

mismatch 10^{-8} 10^{-3}

yes

- **5' → 3' exonuclease**

small fragment repair

No

No

- **when dNTP starved** **3' → 5' pyro-phospholysis**



yes

yes

yes

- **mutation**

lethal

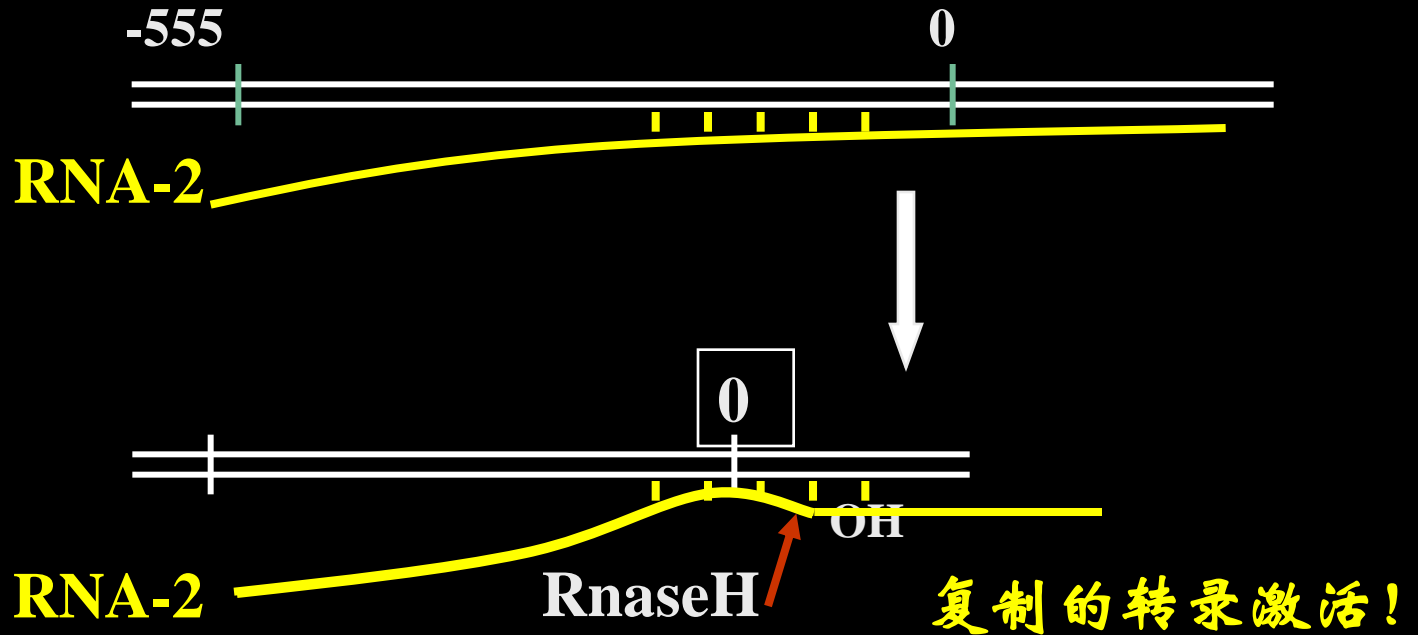
lethal

10-20mole. / E.coli

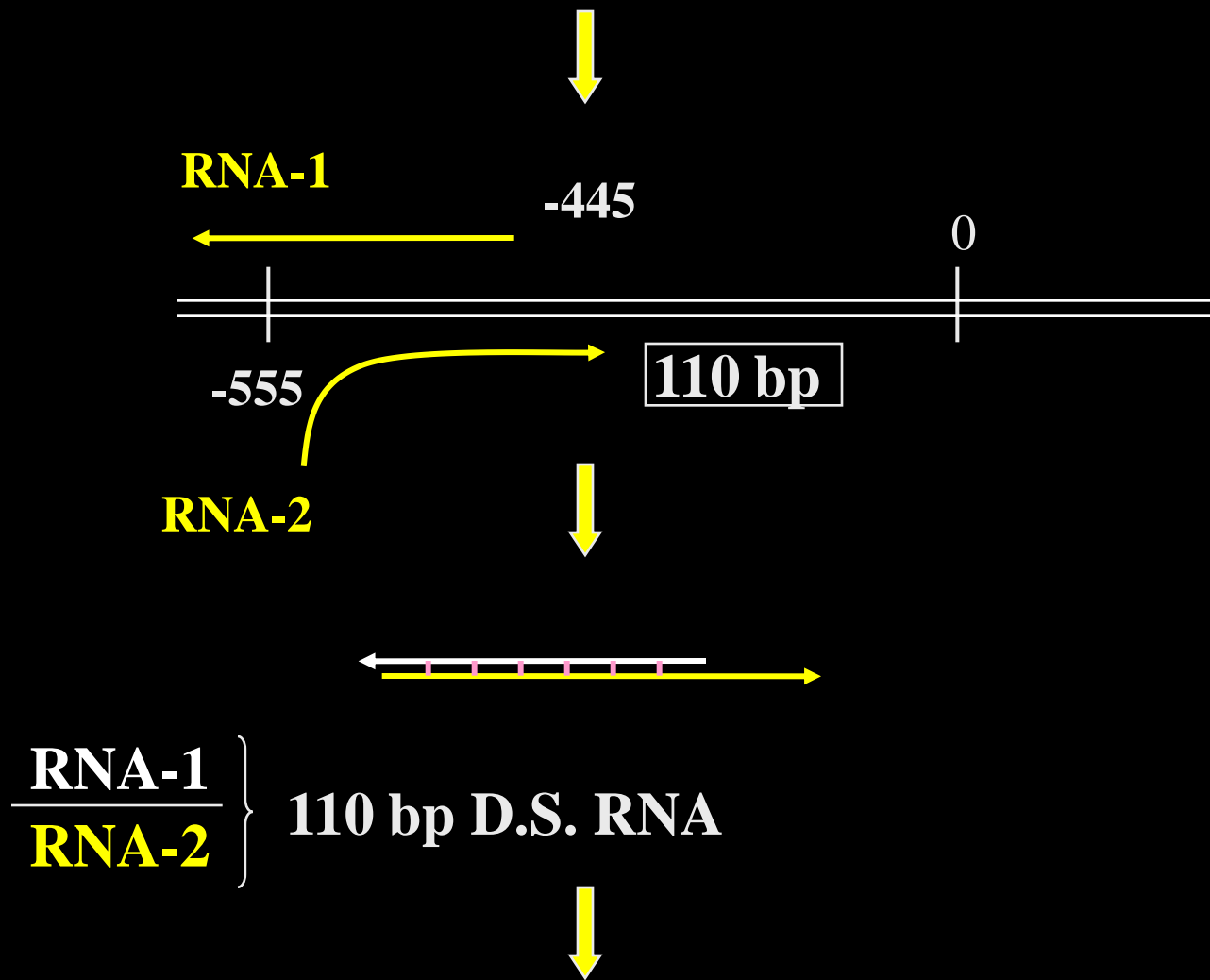
3.5.2 DNA复制的调控

e.g. ColEI plasmid (15-20)

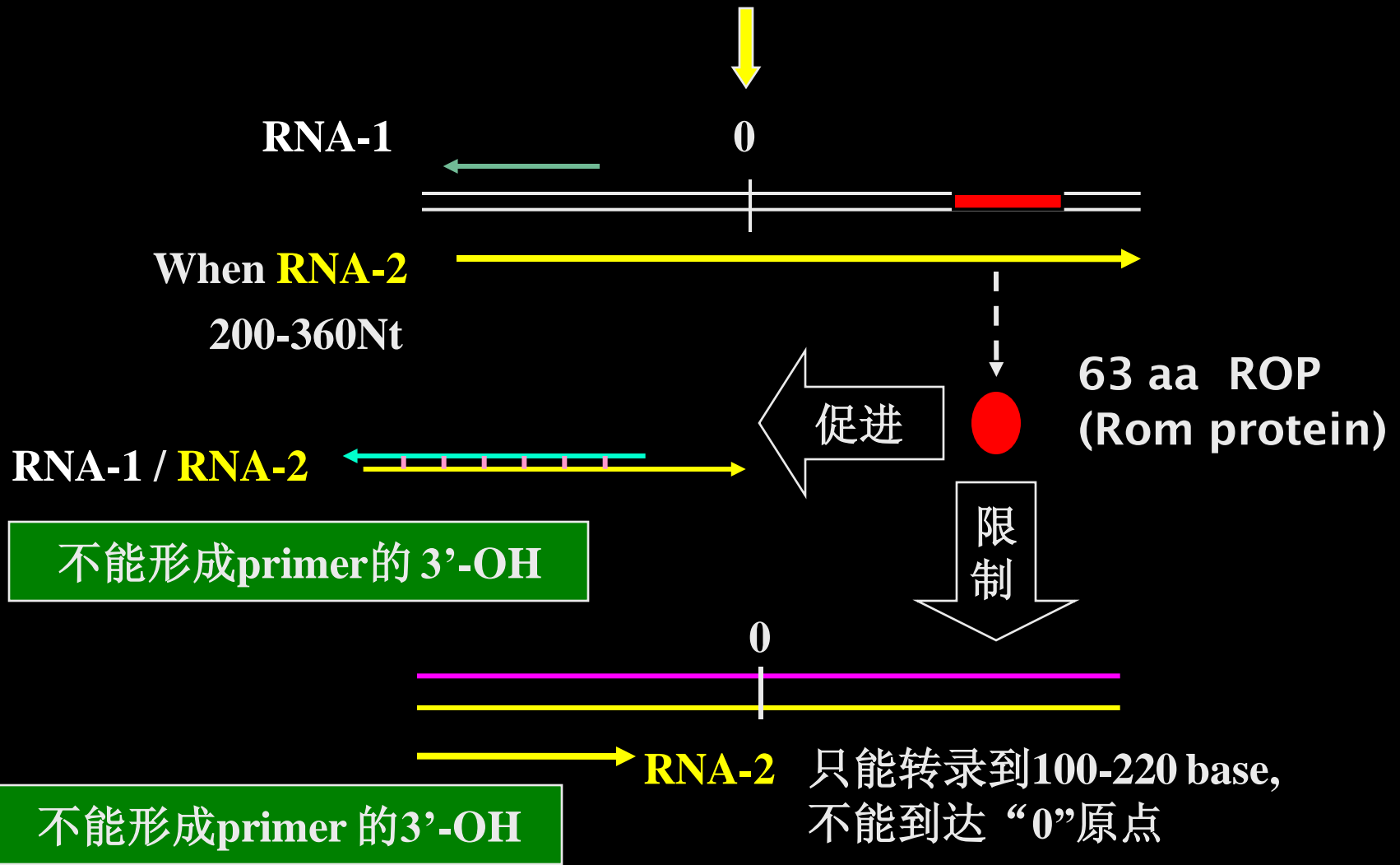
- Repressor model
 - Antisense RNA model
 - **RNA-2**
- } Negative control
- Positive control**



DNA / **RNA** primer for DNA replication



RNase H 不能识别D.S. RNA-1/RNA-2**,
不能通过转录激活形成primer 的3'-OH**



Rom gene expression

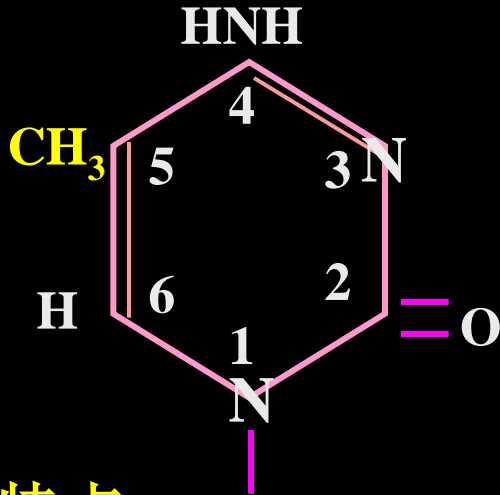
RNA-1 / RNA-2 D.S. repression

ColEI plasmid (15-20 copies)

以负控制为主的调控方式防止过度复制

3.5.3 DNA的甲基化 (Methylation) **(m⁵C in Eukaryote)**

m⁵C

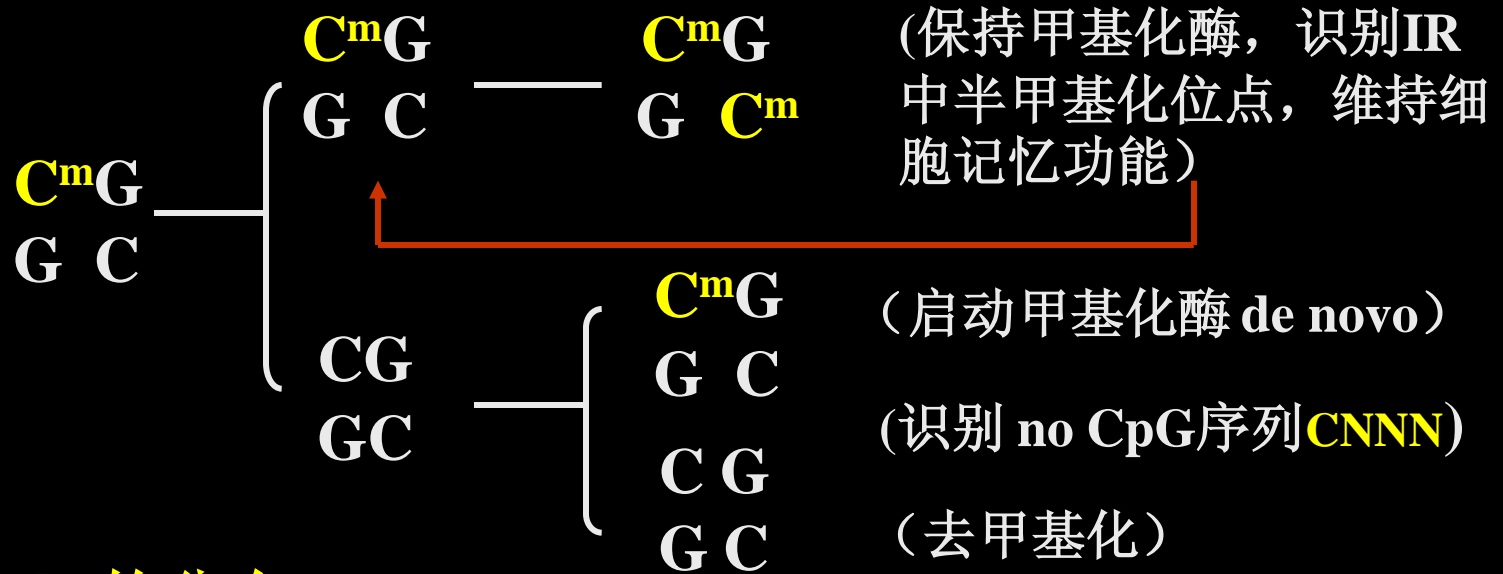


a) DNA中m⁵C的特点

- 对MspI, HpaII 位点的高频修饰

	MspI	HpaII
C^mC GG	-	-
C C^m GG	-	+

● m^5C 的复制



● m^5C 的分布

含CG序列	HpaII	CC^mGG	;	MspI	C^mCGG
	HhaI	GC^mGC	;	ThaI	C^mGC^mG
	Hea	$GG CC^m$;	PstI	C^mTGCAG

● **Animal**

70% of CG seq.

Plant

CNG seq. of Nuclei DNA
(mtDNA, cpDNA no **m⁵C**)

● **Eukaryote**

High repetitive seq. → frequent **m⁵C**

Structure gene → rare **m⁵C**

Cluster gene → frequent **m⁵C**

Expressing gene → rare **m⁵C**

Closed gene → frequent **m⁵C**

(in GC island of promoter)

b) Function of m⁵C

• m⁵C 的不足 → 基因表达相关

m⁵C 的丰富 → 基因关闭相关

m⁵C 的程度具有明显的组织，细胞的特异性(时空性)

(Epigenetic, chromatin remodeling 的重要机制.....)

• 细胞分化，组织特化，阶段发育，组织培养过程的脱分化

与m⁵C的相关性

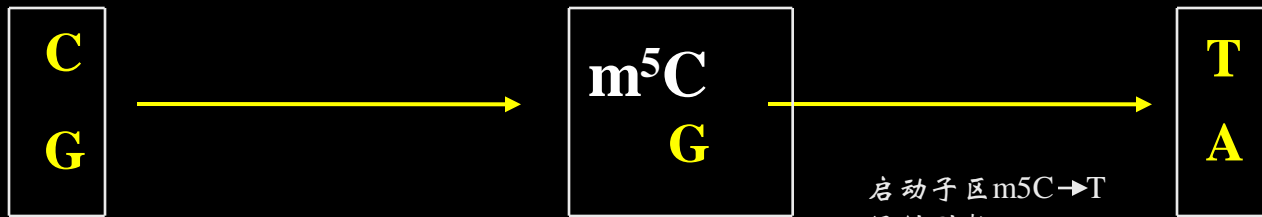
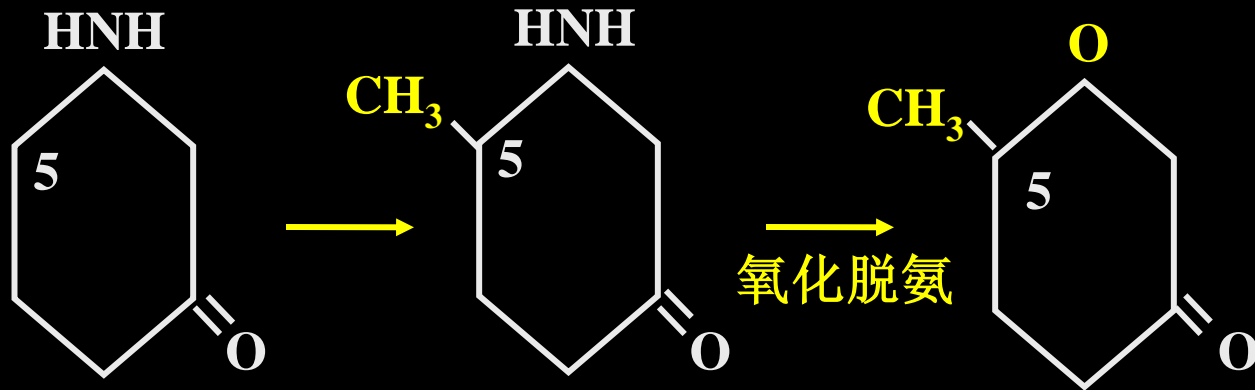
(5-氨脱氧胞苷去甲基化，诱导沉默基因的表达)

• m⁵C --- 甲基化是生物自我保护的机制

• m⁵C --- 使Z-DNA在体内趋于稳定状态

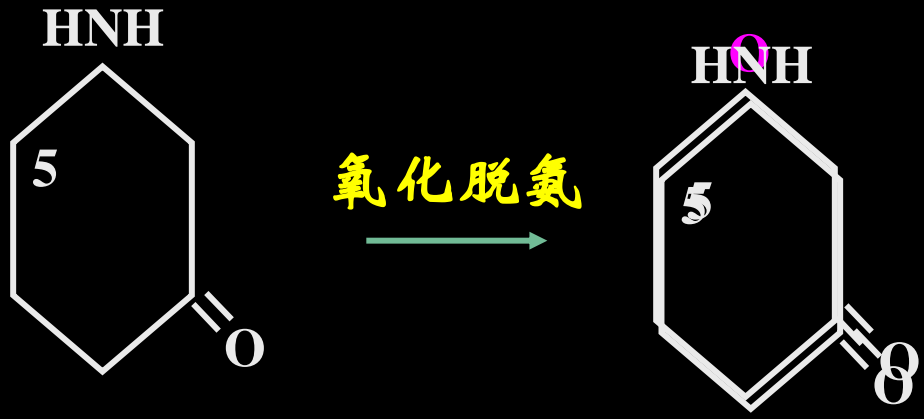
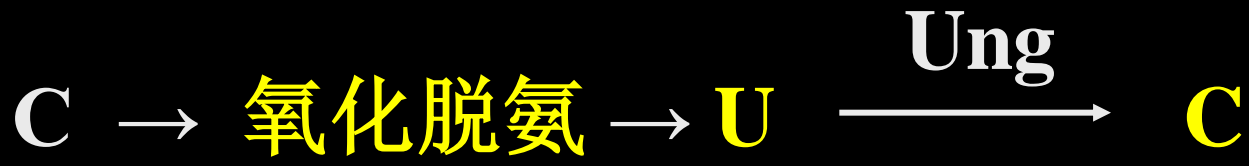
● **m⁵C** 与基因突变的关系

C → **m⁵C** → **T** → gene mutation (癌变)

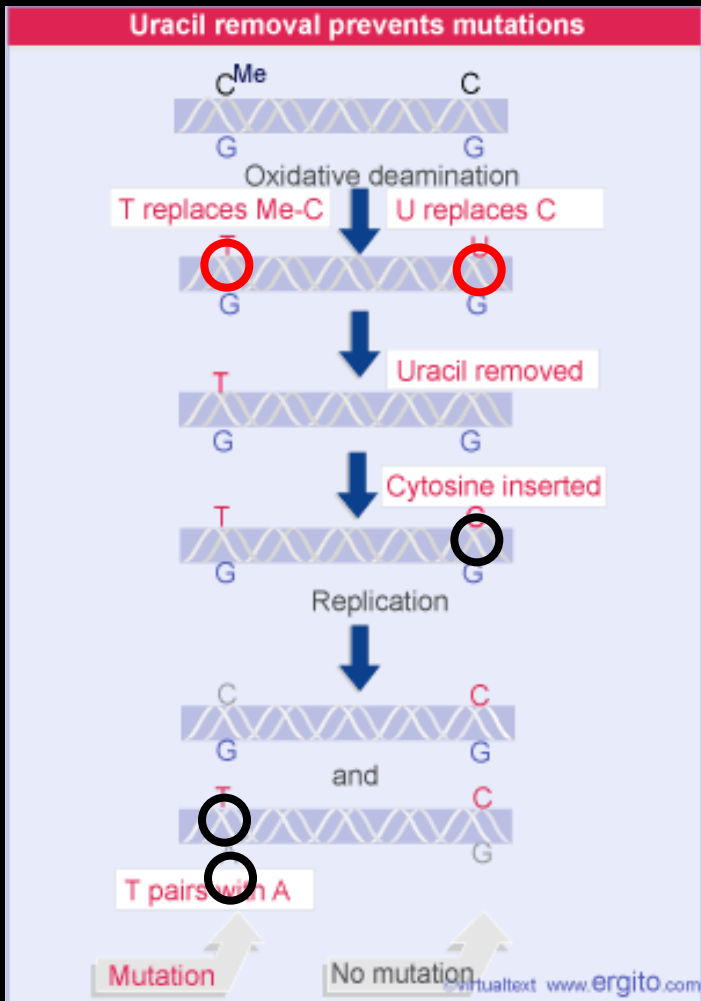


启动子区 m⁵C → T
设计引物

研究基因表达与
性状的相关



避免突变



(来源: 不详)

m^5C 多发生在 CpG 岛, 因此成为真核生物的突变热点

m^5C 占人类基因组 DNA 的 1%, 但突变基因中含有 m^5C 位点的却占 30%

发生氧化脱氨, 导致 $C \rightarrow U$

$m^5C \rightarrow T$

但错配修复系统

倾向于将 G/U 还原成 G/C

减少突变

倾向于将 G/T 校正为 A/T

形成突变

c) Eukaryote

Repetitive seq. high frequent mathylated

PstI m⁵CTGCAG

Sau3A GATm⁵C

Single structure gene rare mathylated

Total DNA digested by PstI or Sau3A

