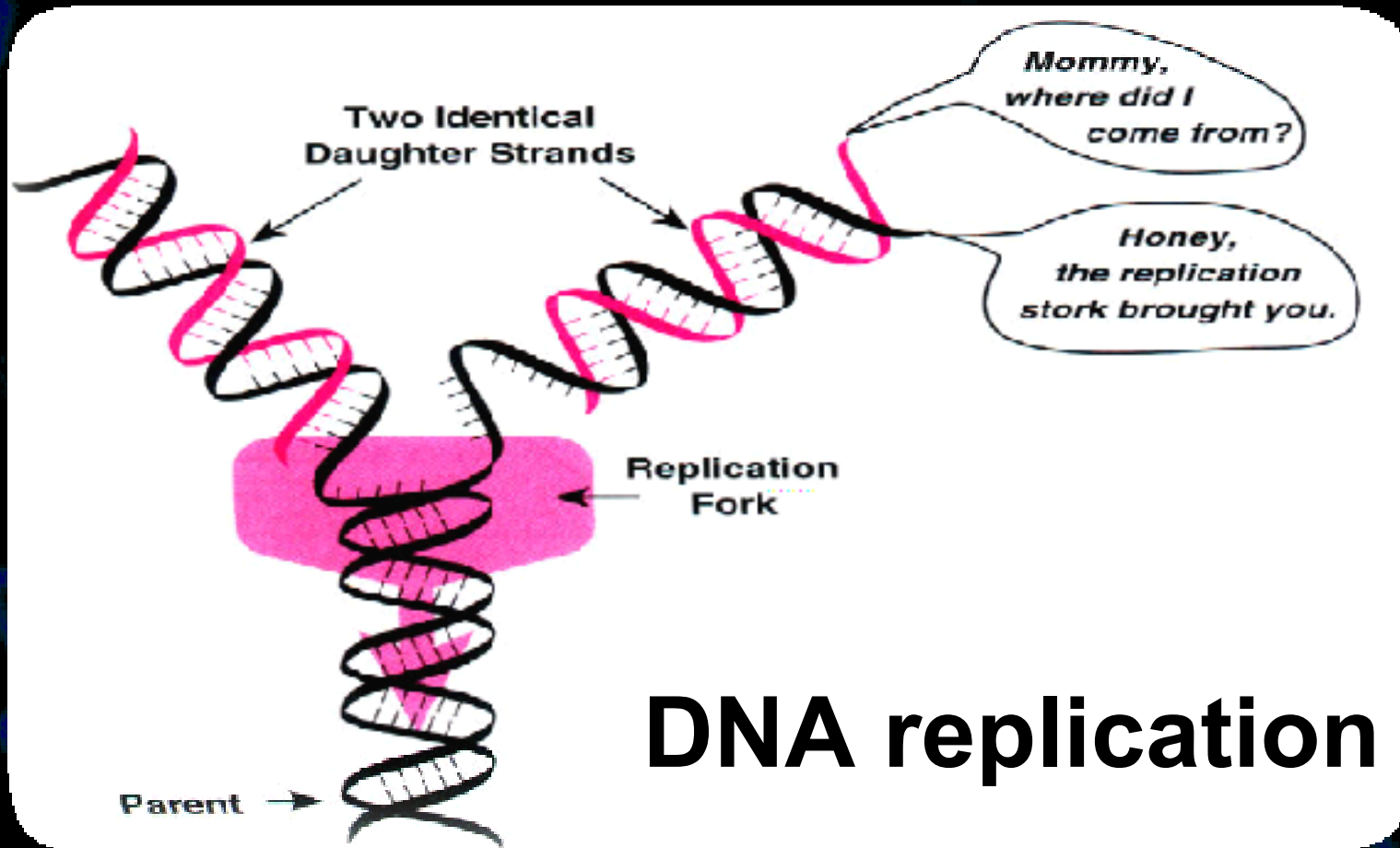
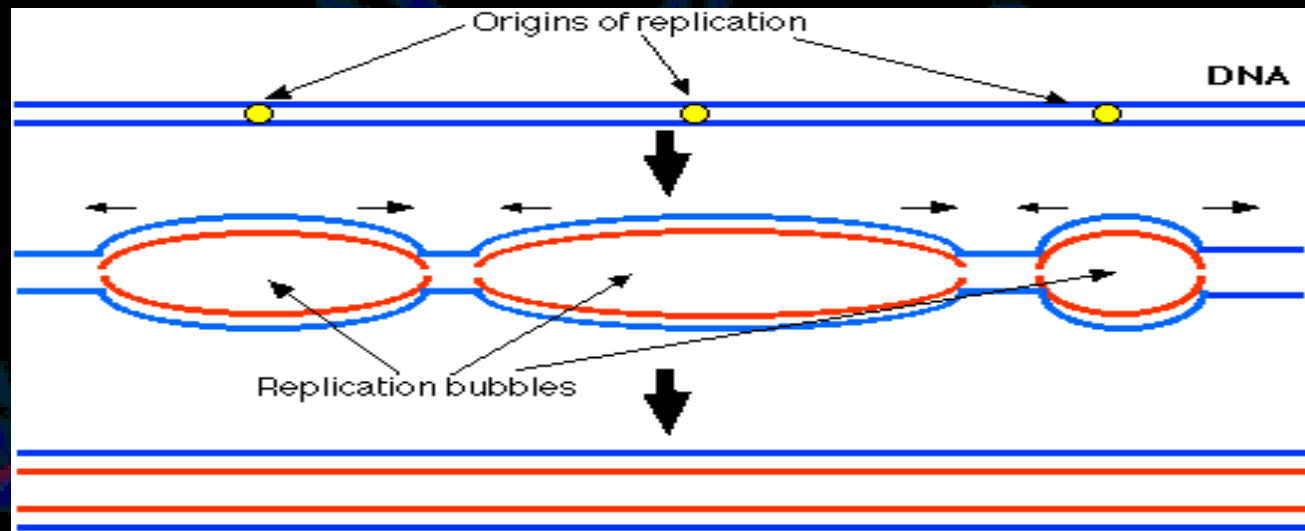


Molecular Biology (三)



Replication bubble

基因轉錄由啟動子附近開始，朝單方向進行；
DNA複製是從複製起點開始，朝雙方向進行。
複製時，兩個叉口合起來像一個小泡，稱之
為複製泡(replication bubble)。



Facts to be considered

Genetic material needs to be reproduced accurately.

Each of the parental strand can act as a template for the synthesis of a complementary daughter strand.

There are several facts needed to be considered :

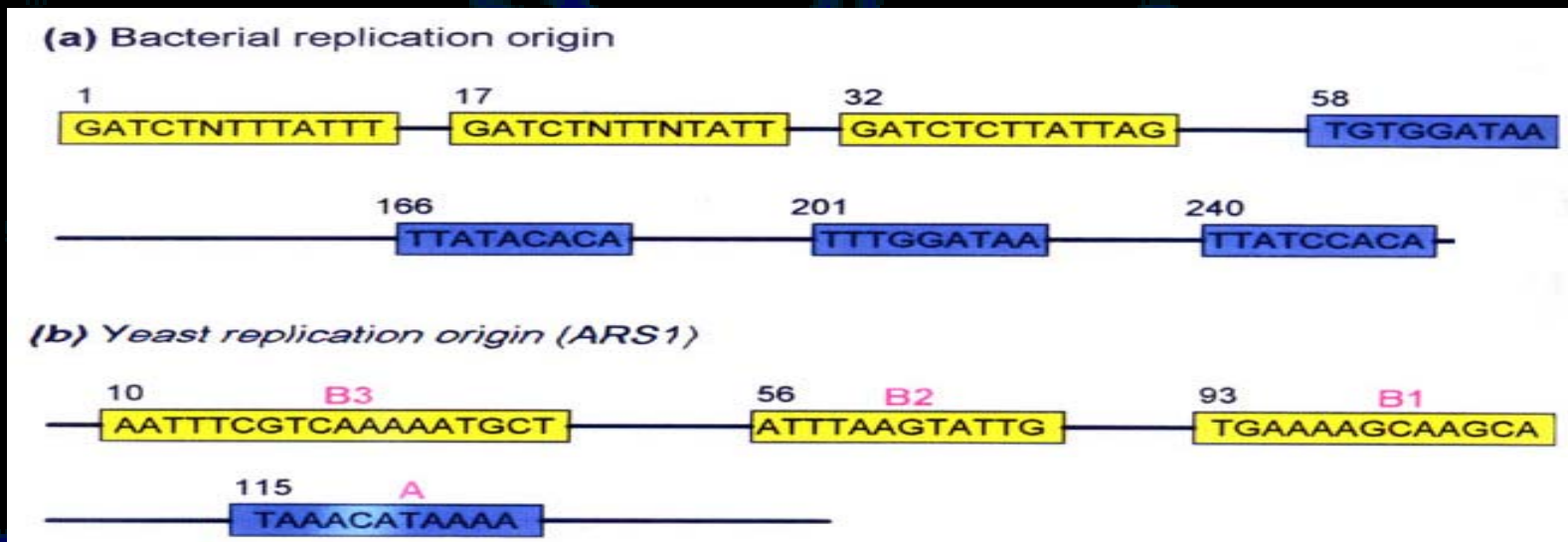
Where is the replication origin

What energy to unwind the double helix

What safeguarding system to prevent replication errors

Replication origin (1)

- 大腸桿菌複製起點的辨認序列涵蓋250 bp左右，包含四個相似的 9-mers (由九個鹼基對組成)和三個相似的13-mers。
 - A、B1、B2 及 B3。A是絕對必要；比較許多個 ARS，發現A的一致序列為 (T/A)AAATA(T/C)AAA(T/A)。B1、B2 及 B3 可以增強啟動複製的效率。
- ARS(autonomously replication sequence)：酵母菌的複製起點。



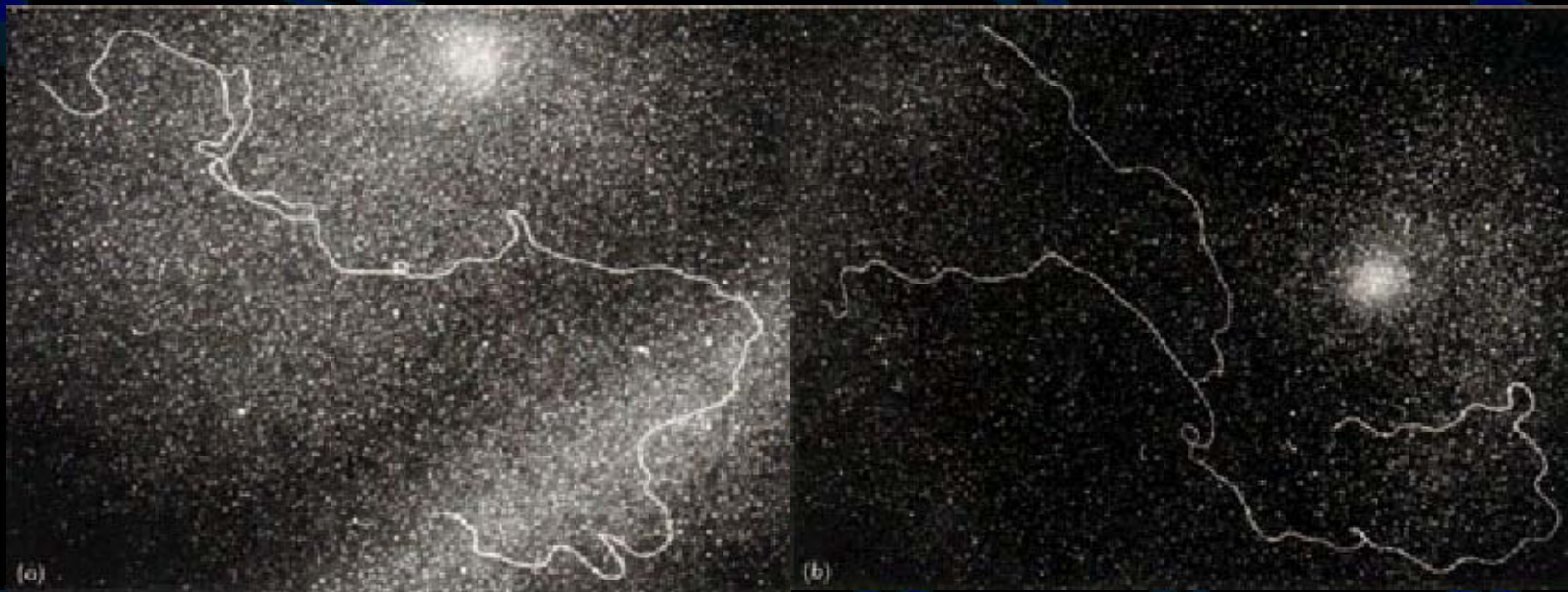
Replication origin (2)

哺乳動物的複製起點較不易從實驗決定。
根據電腦分析結果，其一致序列是 W A W
T T D D W W W D H W G W H M A W T T
(W = A 或 T ; D = A 或 G 或 T ;
H = A 或 C 或 T ; M = A 或 C) 。

各種生物的複製起點皆含有很多個 A 或 T 。這是因為雙股 DNA 裡，A 和 T 只用兩個氫鍵結合，C 和 G 用三個氫鍵結合。所以雙股中含有很多 A 或 T 的區域較容易被解開。

Replication origin (3)

The replication always starts at a point and moves in bidirections generating eye θ -shaped intermediates that convert to Y-shaped molecules. Finally, they separate as two sets of double stranded DNA.



Classification of DNA polymerase

複製時主要的DNA polymerase：

DNA polymerase III

DNA polymerase I

DNA polymerase	δ (III)	α (I)	ε (II)	β	γ
Location	Nuclear	Nuclear	Nuclear	Nuclear	Mitochondrial
Function	Elongation of leading and lagging strand synthesis	Remove primers	Repair	Repair	Replication

Contents of replication

活性因子(Active factor)

連接酶 (Ligase)

引發酶 (Primase)

解螺旋酶 (Helicase)

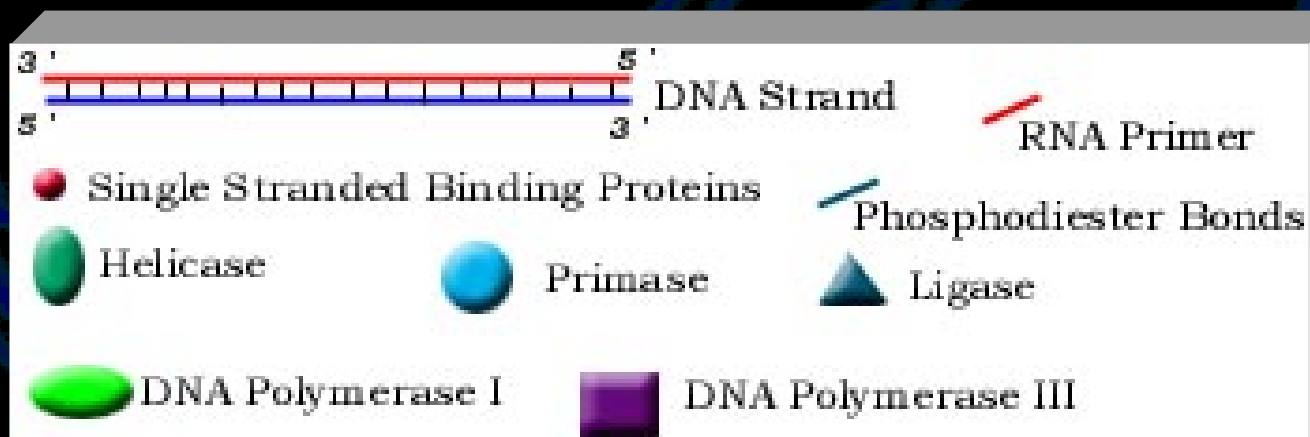
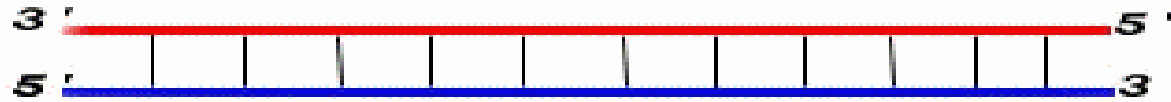
端粒酶 (Telomerase)

單股DNA結合蛋白質 (SSB)

拓撲異構酶 (Topoisomerase)

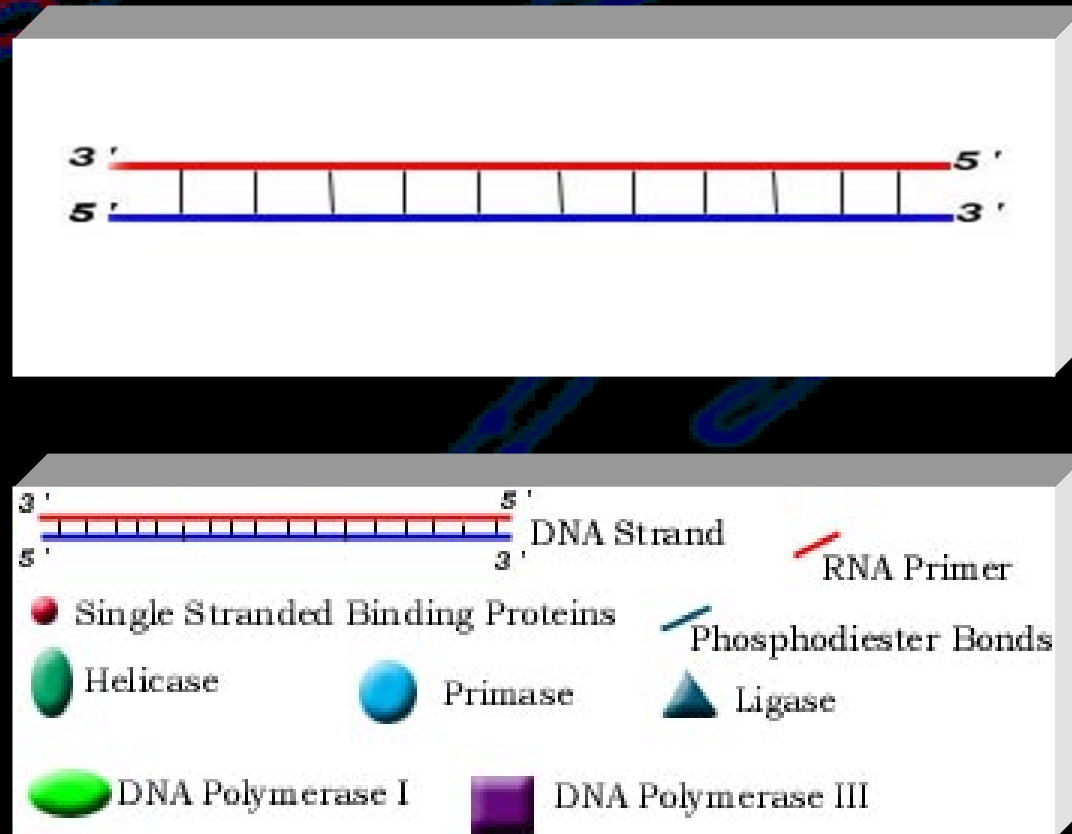
DNA聚合酶 (DNA polymerase)

All steps of DNA replication



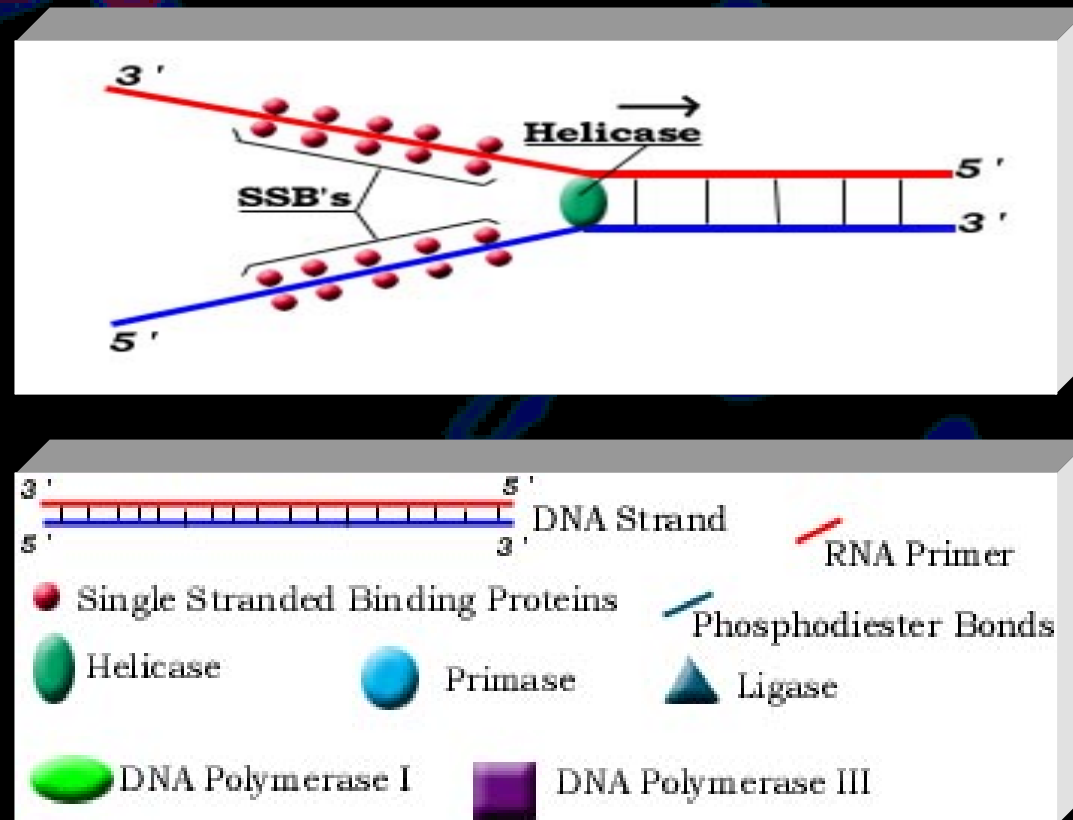
Steps of DNA replication

(A) DNA strand



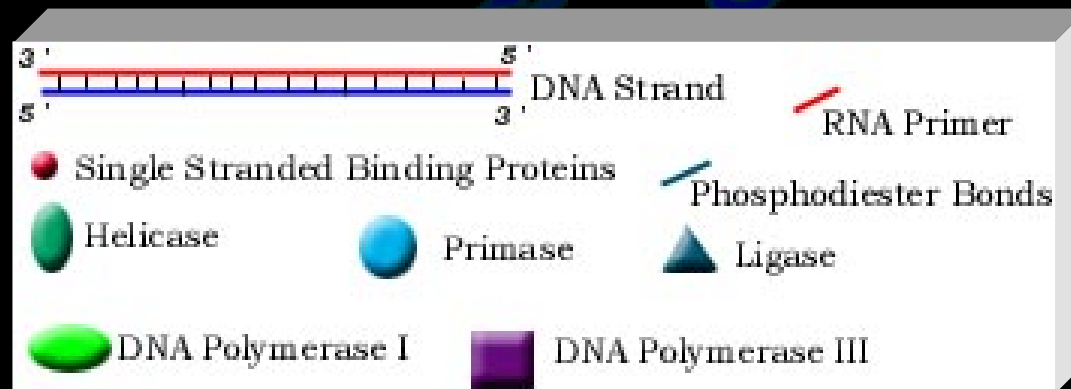
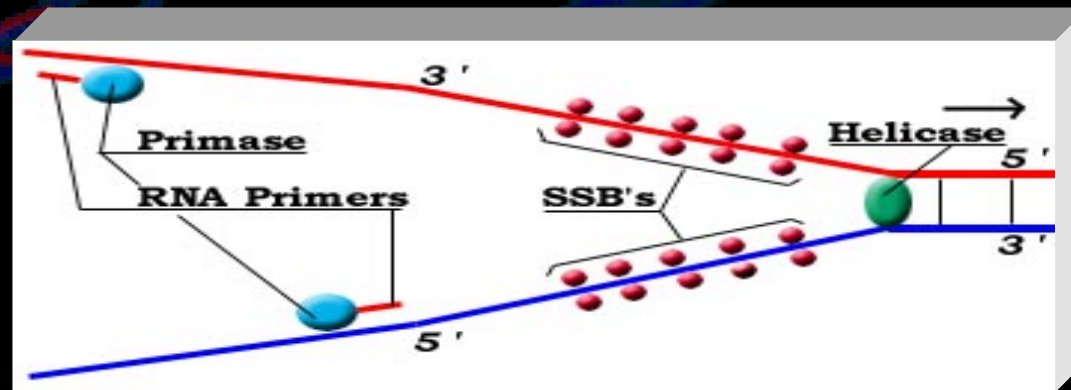
Steps of DNA replication

(B) Helicase unwind the double helix and SSB binding on DNA



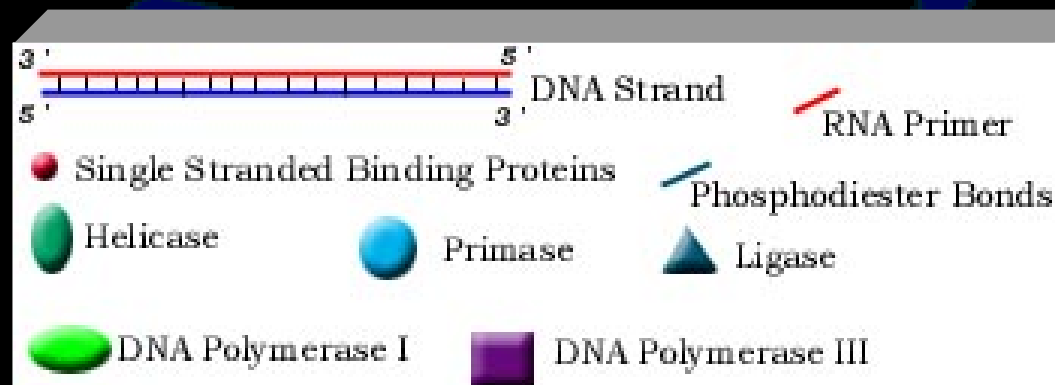
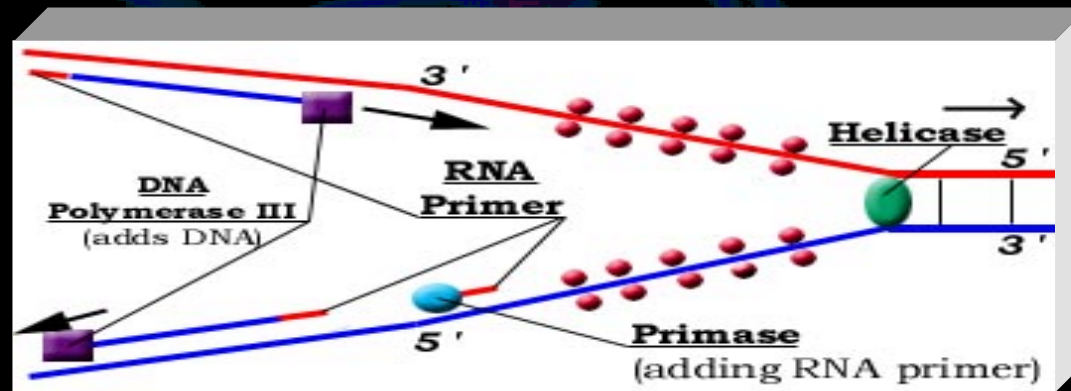
Steps of DNA replication

(C) Primase synthesizes primers



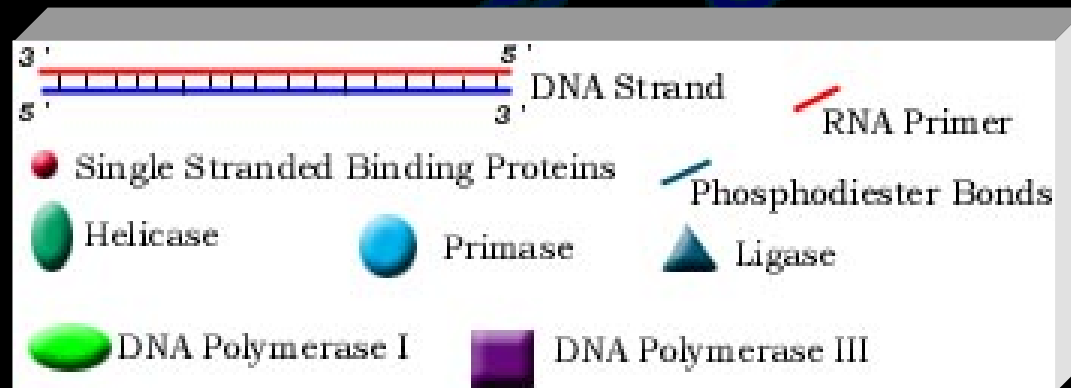
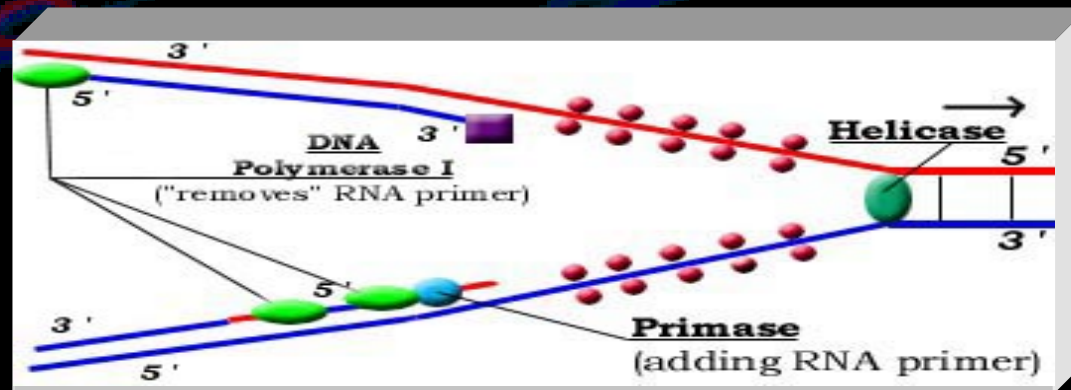
Steps of DNA replication

(D) DNA polymerase III elongates primers with new DNA



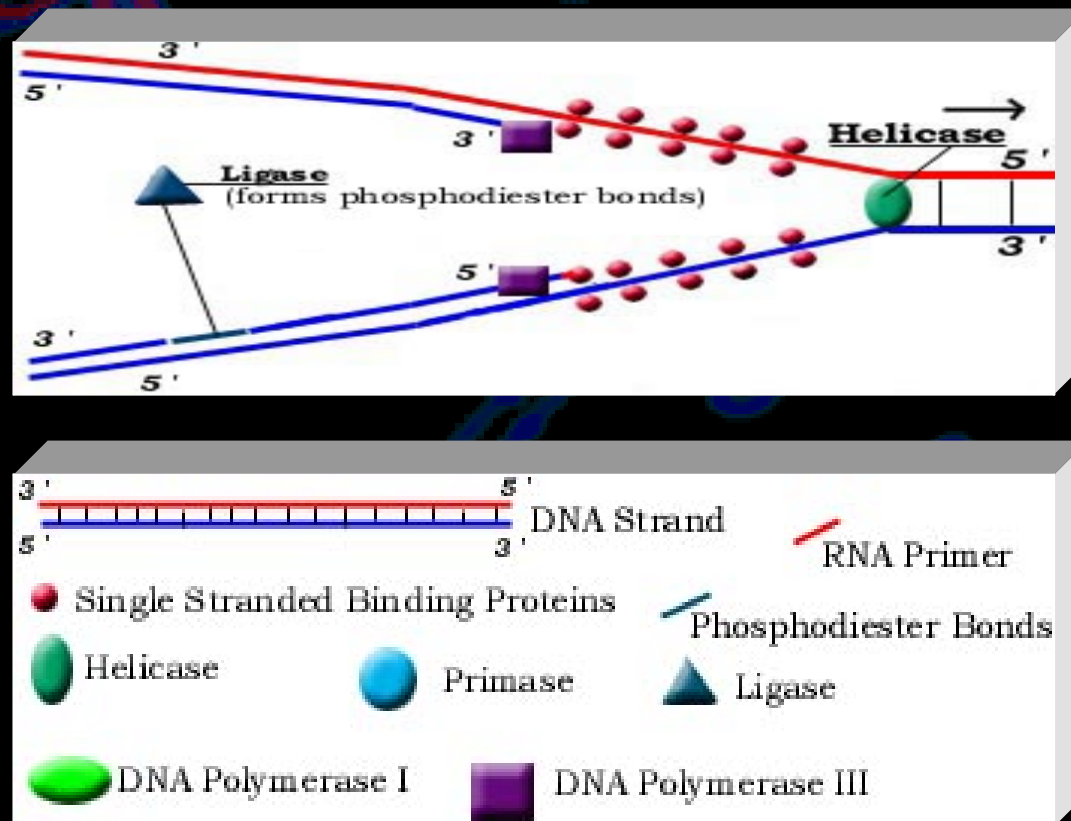
Steps of DNA replication

(E) DNA polymerase I remove primers



Steps of DNA replication

(F) Ligase forms phosphodiester bonds and joins adjacent fragments



Function of telomerase

端粒酶的功能可解決『末端複製問題』

末端複製問題(end-replication problem)

線性DNA中做為落後股(lagging strand)
的模板股最末端將不可能被複製

Telomere

端粒 (telomere)：真核生物的染色體末端

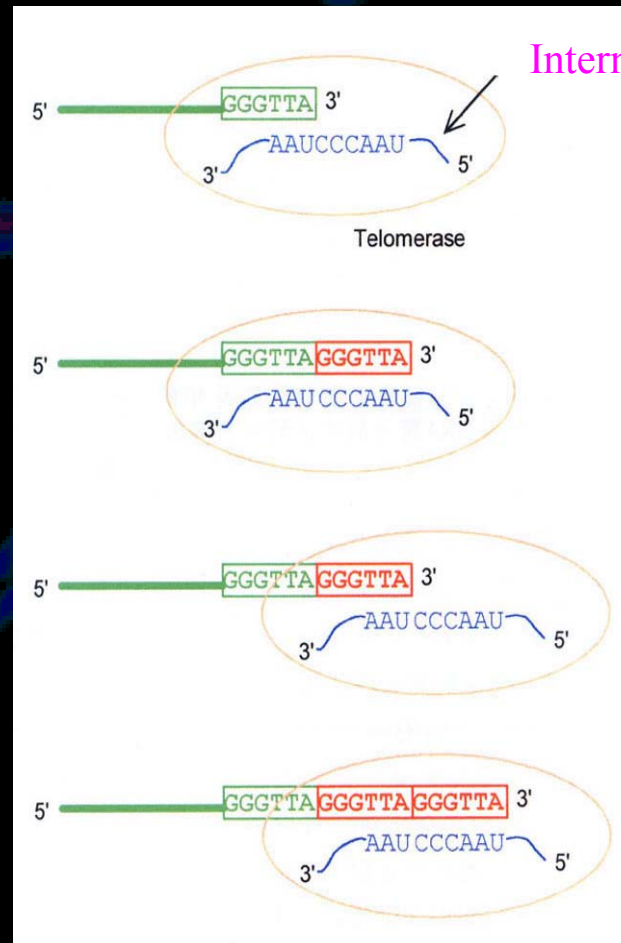
其特定的核苷酸序列具有兩種功能：

- 維持染色體的完整性——
若無端粒，兩個染色體末端將可能融合一起
- 解決末端複製問題——
端粒酶能與端粒作用，延伸其長度

Mechanism of solves end-replication problem



端粒酶包含一段與端粒序列互補的RNA，可延伸較長一股(即複製時落後股的模板股)。引發酶及DNA polymerase 再依照合成落後股方式由5'~3'方向延伸另一股。



Internal RNA template

DNA synthesis

Telomerase translocation

DNA synthesis

Topoisomerase

複製時，部分螺旋被解開後，各種力的平衡受到影響，於是會產生扭轉的超螺旋(supercoil)結構。

Topoisomerase 的主要功能是解開複製時所可能產生的扭曲和纏結。

Topoisomerase可分兩類：

- Topo I：切斷並連接DNA的其中一股
- Topo II：切斷並連接DNA的雙股

Topo I & Topo II 皆能解開超螺旋結構

Topo I 一次能解開一個結，不需外界能量

Topo II一次能解開兩個結，但需要藉助於ATP水解的能量

