BHARATHIDASAN UNIVERSITY

Tiruchirappalli 620024
Tamilnadu, India

Program: M.Sc., Microbiology

Course Title : Microbial Genetics & Molecular Biology

Course Code: 24MICCC3

Unit III: DNA replication

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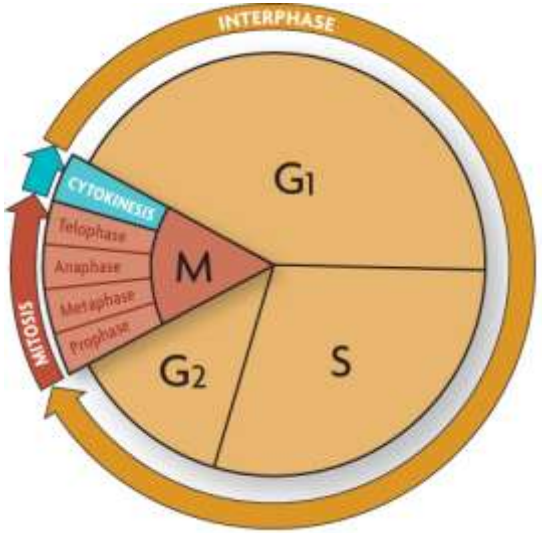
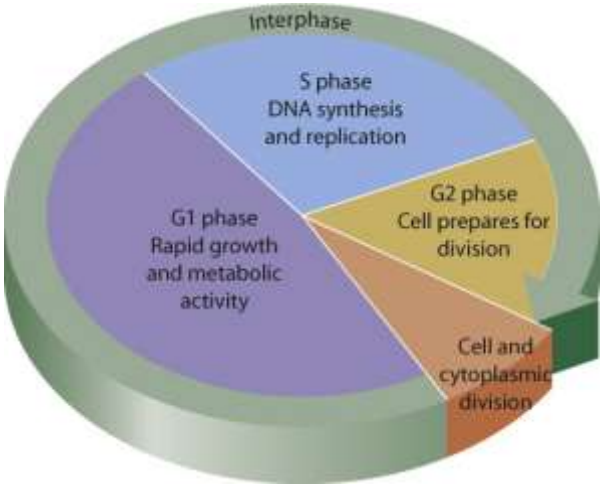
DNA REPLICATION

DNA replication copies the genetic information of a cell.

Replication copies the genetic information.

- A single strand of DNA serves as a template for a new strand.
- The rules of base pairing direct replication.
- DNA is replicated during the S (synthesis) stage of the cell cycle.
- Each body cell gets a complete set of identical DNA.



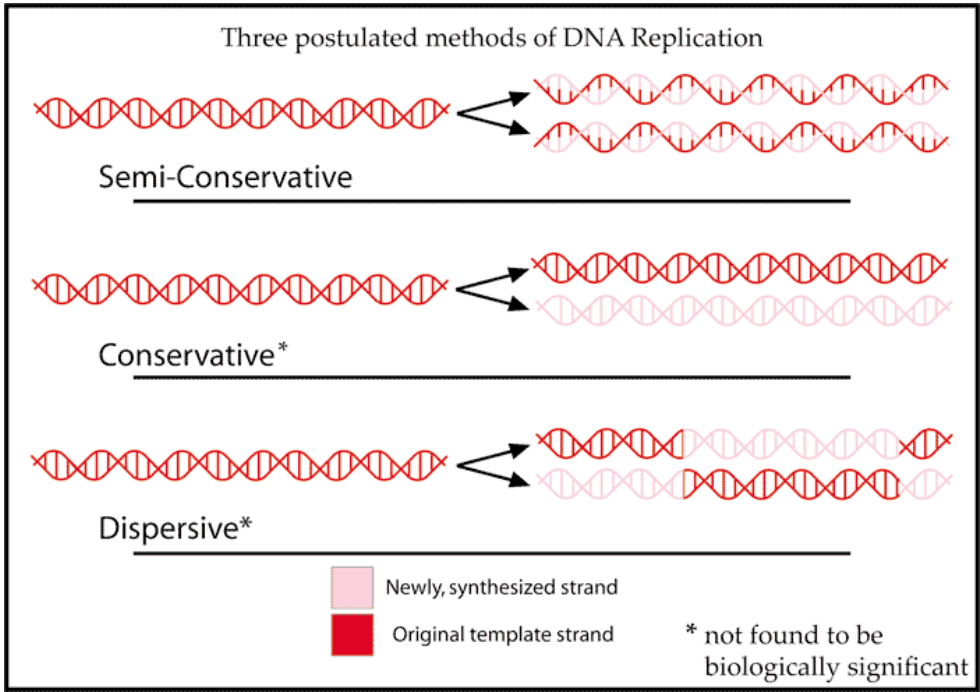


DNA Replication

Matthew Meselson & Franklin Stahl, 1958 investigated the process of DNA replication considered 3 possible mechanisms:

- **Conservative model**
- **Semiconservative model**
- **Dispersive model**

The conservative model results in one new molecule and conserves the old. The **semi-conservative replication** model results in two hybrid molecules of old and new strands. The dispersive model results in hybrid molecules with each strand being a mixture of old and new strands.



Meselson and Stahl Determine the Mechanism of DNA Replication

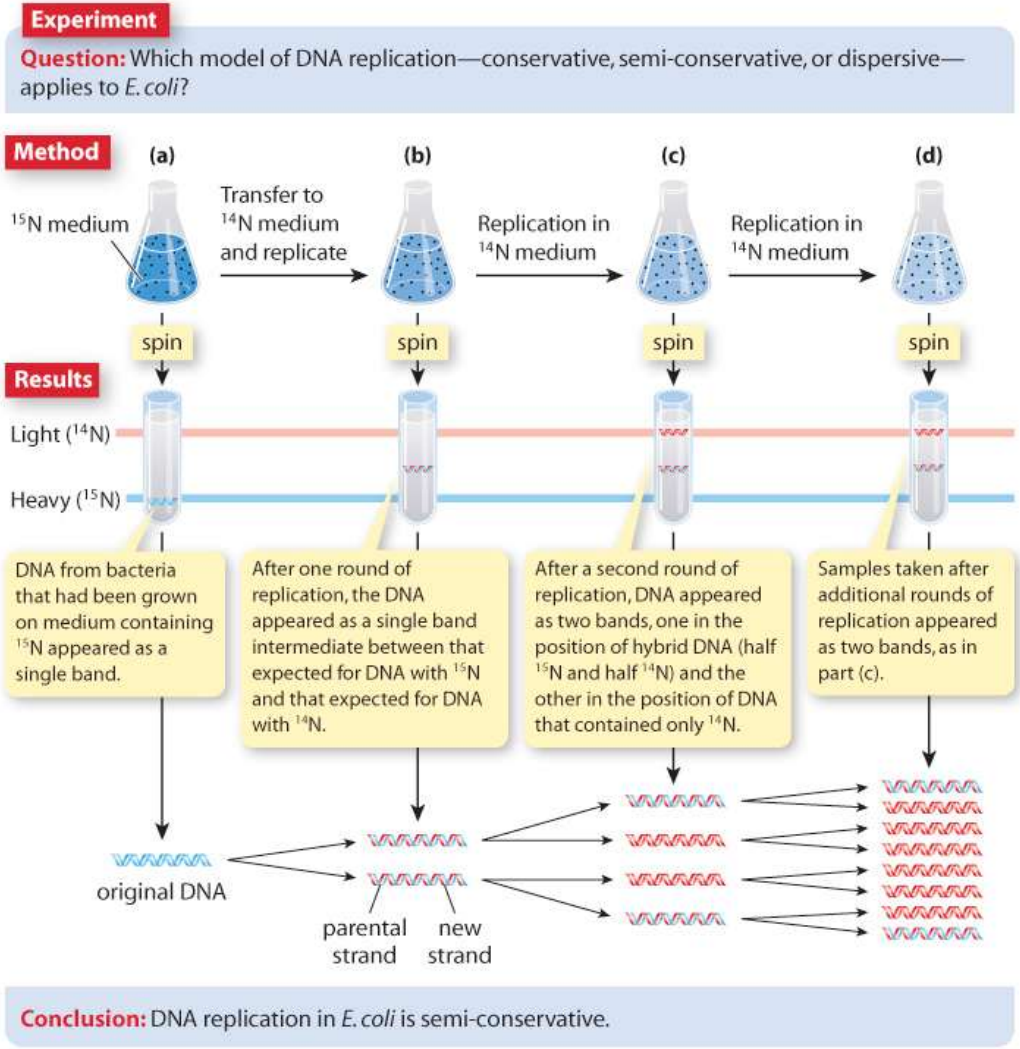
Matthew Meselson and Franklin Stahl reasoned that they could test the models if they could distinguish between parent and daughter strands of DNA. They used two different isotopes of nitrogen to label the DNA: "light" ^{14}N and "heavy" ^{15}N. These isotopes were chosen for two reasons:

- Nitrogen is a component of DNA and would be incorporated into newly synthesized daughter strands.
- "Light" and "heavy" forms of nitrogen would allow separation of different strands of DNA based on how much isotope was present. DNA with the "heavy" nitrogen would be more dense than DNA with "light" nitrogen.

Dr. Matthew Meselson

Dr. Franklin Stahl

Meselson and Stahl Determine the Mechanism of DNA Replication

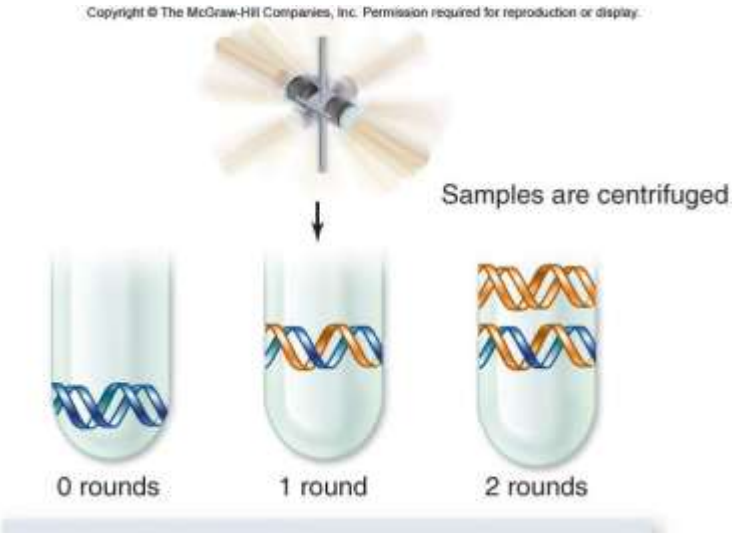


At the 0 time point: all the DNA had heavy ^{15}N nitrogen

After 1 round: the DNA was a hybrid molecule, with an intermediate location

After 2 rounds: two molecules were seen:
one that was hybrid, and one that was the lighter ^{14}N DNA molecule.

Conclusion: Semiconservative replication



DNA Replication

Meselson and Stahl concluded that the mechanism of DNA replication is the **semiconservative model**.

Each strand of DNA acts as a template for the synthesis of a new strand.

DNA Replication

DNA replication includes:

- **Initiation** – replication begins at an **origin of replication**
- **Elongation** – new strands of DNA are synthesized by **DNA polymerase**
- **Termination** – replication is terminated differently in prokaryotes and eukaryotes

Prokaryotic DNA Replication

The chromosome of a prokaryote is a circular molecule of DNA.

Replication begins at one **origin of replication** and proceeds in both directions around the chromosome.

- Origins of replications usually are rich in Adenine and Thymine

Enzymes of Prokaryotic DNA Replication

- The double helix is unwound by the enzymes helicase , DNA topoisomerase, and DNA gyrase
  - SSBP (single stranded binding protein) helps keep strands separated
- DNA polymerase III (pol III) is responsible for most of DNA synthesis
  - Adds nucleotides to the 3’ end of the daughter strand of DNA; DNA synthesis is from 5' to 3'
  - Requires RNA primers as a guide for synthesis
- RNA primers are made by the enzyme primase

Enzymes of Prokaryotic DNA Replication

- DNA polymerase I: involved in proofreading and DNA repair
- DNA ligase: involved in connected ends of replicated DNA together

The Problem of Overwinding

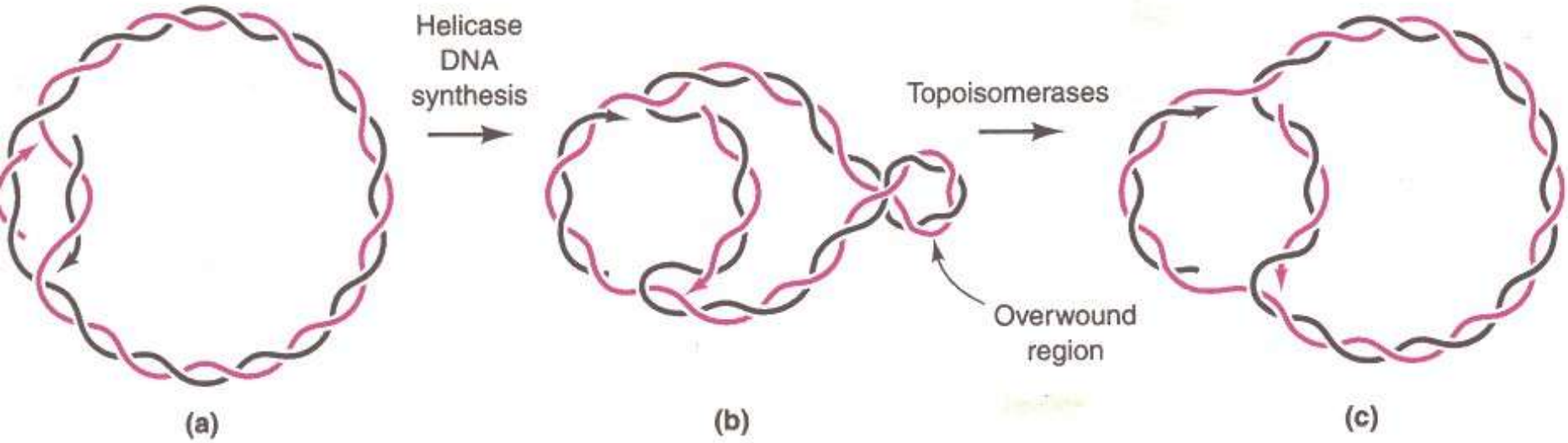


Figure 7-4 Relaxation of DNA overwinding by topoisomerases. DNA replication is shown after it has begun on a circular DNA molecule. In this example a replication fork is working at both sides of the growing "bubble" (a). As the replication bubble grows (b), an overwound region appears ahead of the replication bubble. Through the action of topoisomerases the overwound region is relaxed (c). Illustration provided by C. Ullsperger, A. Vologodskii, and N. Cozzarelli.

Topoisomerase Type I

- Precedes replicating DNA
- Mechanism
  - Makes a cut in one strand, passes other strand through it. Seals gap.
  - Result: induces positive supercoiling as strands are separated, allowing replication machinery to proceed.

Gyrase--A Type II Topoisomerase

- Introduces negative supercoils
- Cuts both strands
- Section located away from actual cut is then passed through cut site.

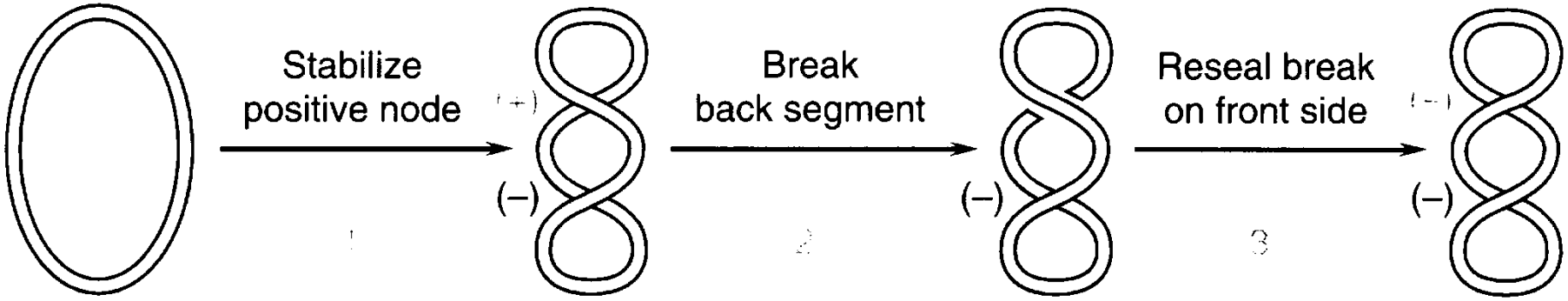
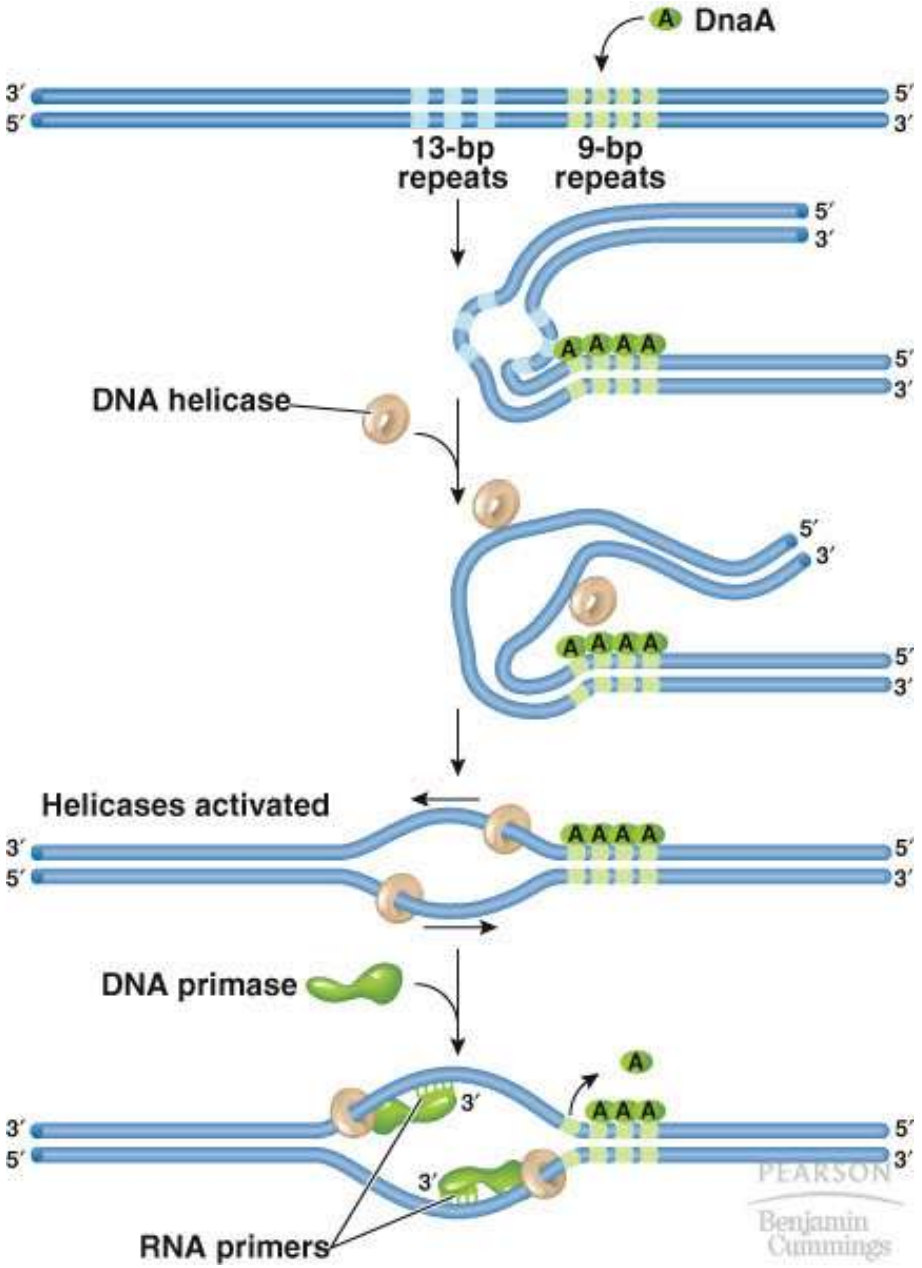
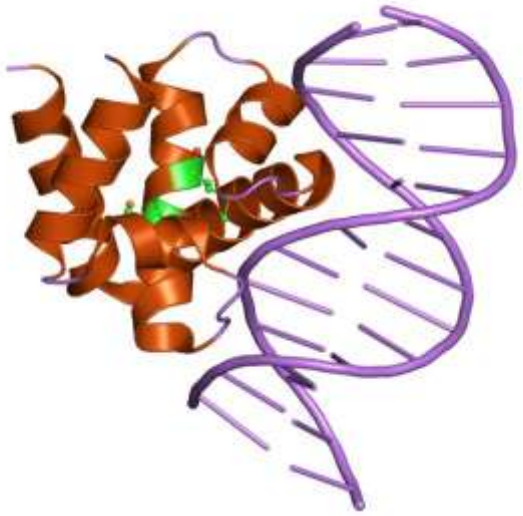


Figure. Model of the formation of negative DNA supercoils by DNA gyrase.

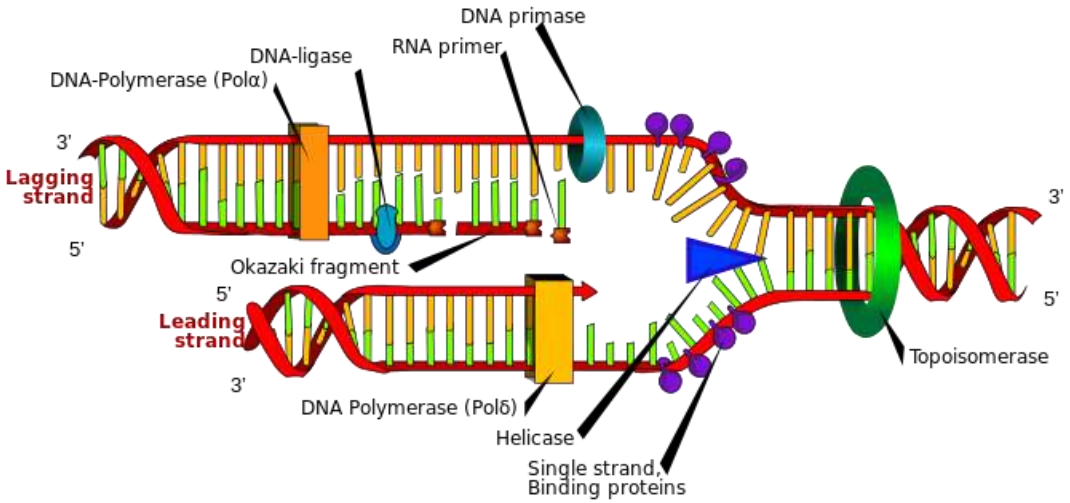
Initiation of replication in *E. coli*. The DnaA initiator protein binds to *oriC* (the replicator) and stimulates denaturation of the DNA. DNA helicases are recruited and begin to untwist the DNA to form two head-to-head replication forks.



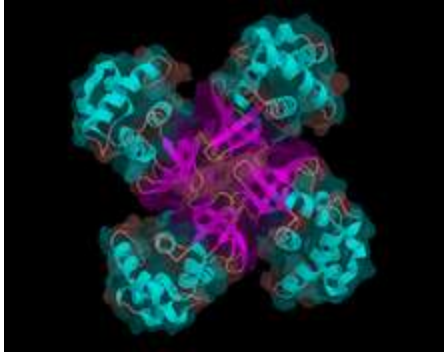
- DnaA – Protein activates initiation of DNA replication in prokaryotes
- Replication initiation factor which promotes the unwinding of DNA at oriC
- Replication begins with active DnaA binding to 9-mer (9-bp) repeats upstream of the oriC
- Binding of DnaA leads to strand separation at the 13-mer repeats
- This binding causes the DNA to loop in preparation for melting open by the helicase DnaB.
- The oriC site in *E. coli* has three AT rich 13 base pair regions (DUEs) followed by four 9 bp regions
- 10 dnaA molecules bind to the 9 bp regions, which wrap around the proteins causing the DNA at the AT-rich region to unwind
- When DNA replication is about to commence, dnaA occupies all of the high and low affinity binding sites



- The denatured AT-rich region allows for the recruitment of DnaB (helicase), which complexes with DnaC (helicase loader)
- DnaC helps the helicase to bind to and to properly accommodate the ssDNA at the 13 bp region; this is accomplished by ATP hydrolysis, after which DnaC is released
- Single-strand binding proteins (SSBs) stabilize the single DNA strands in order to maintain the replication bubble
- DnaB is a 5'→3' helicase, so it travels on the lagging strand. It associates with DnaG (a primase) to form the only primer for the leading strand and to add RNA primers on the lagging strand
- The interaction between DnaG and DnaB is necessary to control the longitude of Okazaki fragments on the lagging strand
- DNA polymerase III is then able to start DNA replication



Helicase



- Motor proteins that move directionally along a nucleic acid phosphodiester backbone, separating two annealed nucleic acid strands (i.e., DNA, RNA, or RNA-DNA hybrid) using energy derived from ATP hydrolysis
- Cellular processes, such as DNA replication, transcription, translation, recombination, DNA repair, and ribosome biogenesis involve the separation of nucleic acid strands that necessitates the use of helicases
- They also function to remove nucleic acid-associated proteins and catalyze homologous DNA recombination
- Metabolic processes of RNA such as translation, transcription, ribosome biogenesis, RNA splicing, RNA transport, RNA editing, and RNA degradation are all facilitated by helicases
- Helicases move incrementally along one nucleic acid strand of the duplex with a directionality and processivity specific to each particular enzyme
- Helicases adopt different structures and oligomerization states
- DnaB-like helicases unwind DNA as donut-shaped hexamers, other enzymes have been shown to be active as monomers or dimers

- Enzymatic helicase action, such as unwinding nucleic acids depends on activation barrier (B)
- Its unwinding progression is largely affected by the sequence of nucleic acids within the molecule to unwind, and the presence of destabilization forces acting on the replication fork
- So far 25 helicases have been well characterized
- Helicases have been classified in 6 major groups (superfamilies) based on the motifs and consensus sequences shared by the molecules
- Helicase - not forming ring structures - superfamily 1 and 2
- - forming ring structures - superfamily 3 to 6
- Alpha Helicase - act on single stranded DNA
- Beta Helicase - act on dsDNA
- Type A Helicase - translocation at 3'to 5' direction
- Type B Helicase - translocation at 5' to 3' direction

Liebowitz Experiment

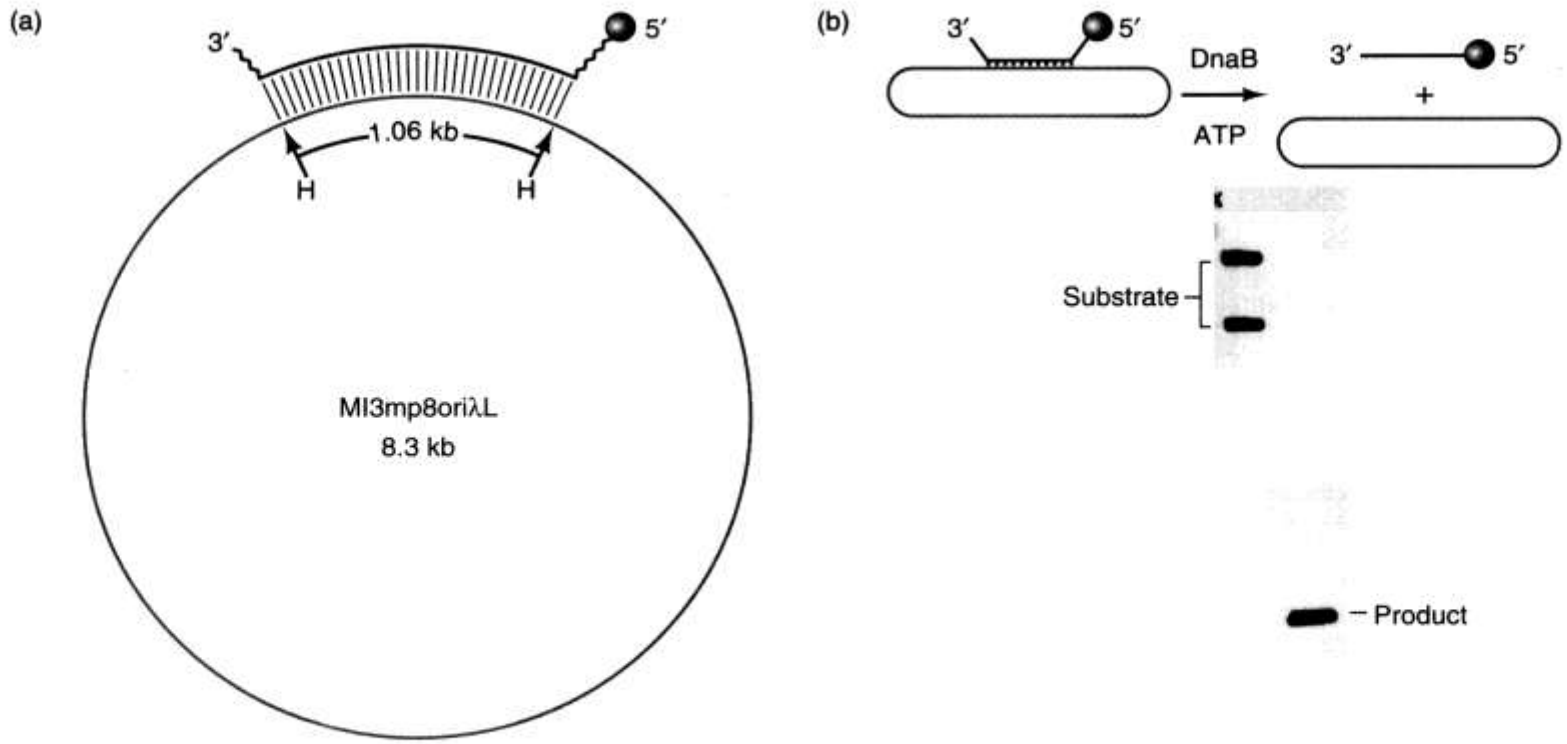
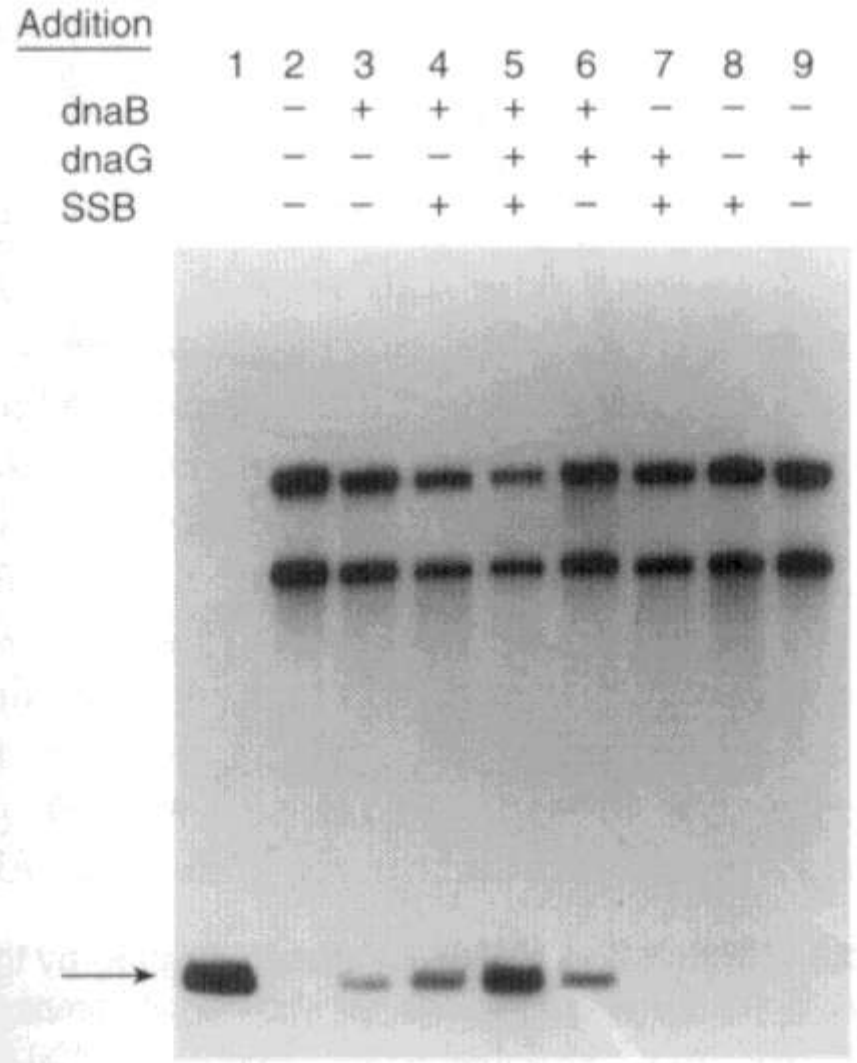


Figure 20.18 DNA helicase assay. (**a**) Substrate. LeBowitz and McMacken made a helicase substrate by ^{32}P-labeling a single-stranded 1.06 kb *Hin*cII DNA fragment (red) at its 5′-end, and annealing the fragment to an unlabeled single-stranded recombinant M13 DNA bearing a complementary 1.06 kb region, bounded by two *Hin*cII sites (H). (**b**) Assay. Top: The dnaB protein, or any DNA helicase, can unwind the double-stranded region of the substrate and liberate the labeled short piece of DNA (red) from its longer, circular partner. Bottom: Electrophoresis of the substrate (lane 1) yields two bands, which probably correspond to linear and circular versions of the long DNA annealed to the labeled, short DNA. Electrophoresis of the short DNA by itself (lane 2) shows that it has a much higher mobility than the substrate (see band labeled "product").

Liebowitz Assay--Results



- What do these results indicate?
- ALTHOUGH PRIMASE (DnaG) AND SINGLE-STRAND BINDING PROTEIN (SSB) BOTH STIMULATE DNA HELICASE (DnaB), NEITHER HAVE HELICASE ACTIVITY OF THEIR OWN

Single Stranded DNA Binding Proteins (SSB)

- Maintain strand separation once helicase separates strands
- Not only separate and protect ssDNA, also stimulates binding by DNA pol (too much SSB inhibits DNA synthesis)
- Strand growth proceeds 5'>>>3'

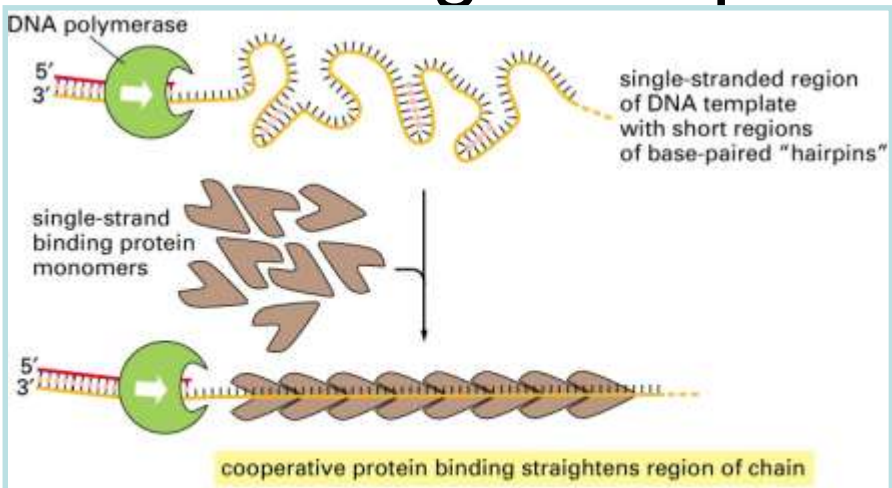
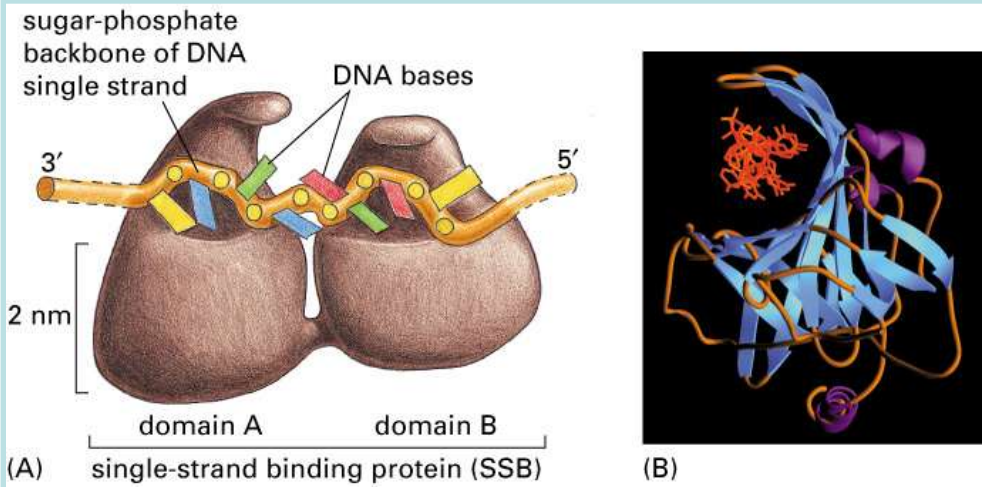


Figure 5–17. Molecular Biology of the Cell, 4th Edition.



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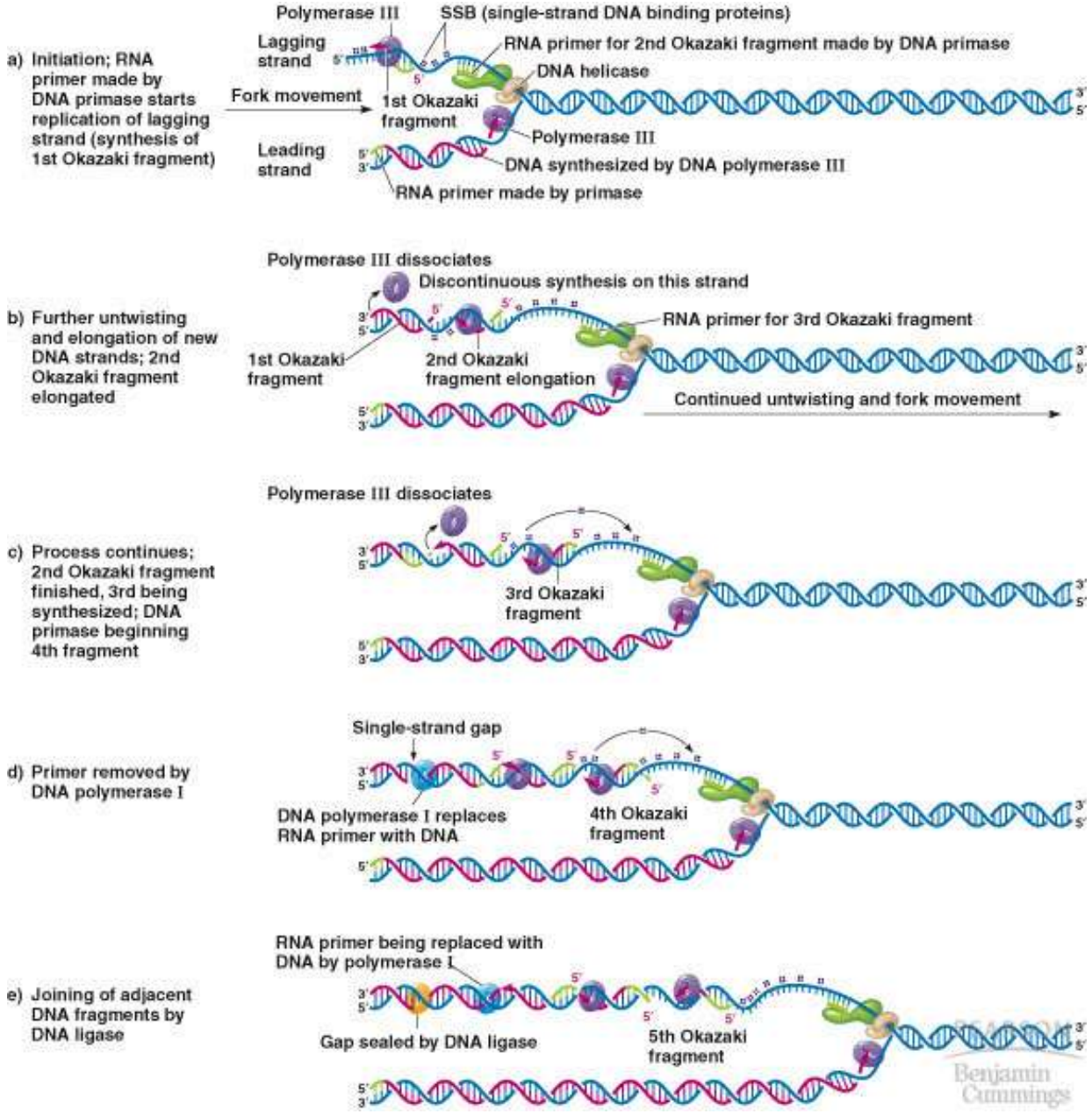

a) Initiation; RNA primer made by DNA primase starts replication of lagging strand (synthesis of 1st Okazaki fragment)
Polymerase III
SSB (single-strand DNA binding proteins)
Lagging strand
RNA primer for 2nd Okazaki fragment made by DNA primase
DNA helicase
Fork movement
1st Okazaki fragment
Polymerase III
Leading strand
DNA synthesized by DNA polymerase III
RNA primer made by primase
b) Further untwisting and elongation of new DNA strands; 2nd Okazaki fragment elongated
Polymerase III dissociates
Discontinuous synthesis on this strand
RNA primer for 3rd Okazaki fragment
1st Okazaki fragment
2nd Okazaki fragment elongation
Continued untwisting and fork movement
c) Process continues; 2nd Okazaki fragment finished, 3rd being synthesized; DNA primase beginning 4th fragment
Polymerase III dissociates
3rd Okazaki fragment
d) Primer removed by DNA polymerase I
Single-strand gap
DNA polymerase I replaces RNA primer with DNA
4th Okazaki fragment
e) Joining of adjacent DNA fragments by DNA ligase
RNA primer being replaced with DNA by polymerase I
Gap sealed by DNA ligase
5th Okazaki fragment
Benjamin Cummings

The DNA Polymerase Family

A total of 5 different DNAPs have been reported in E. coli

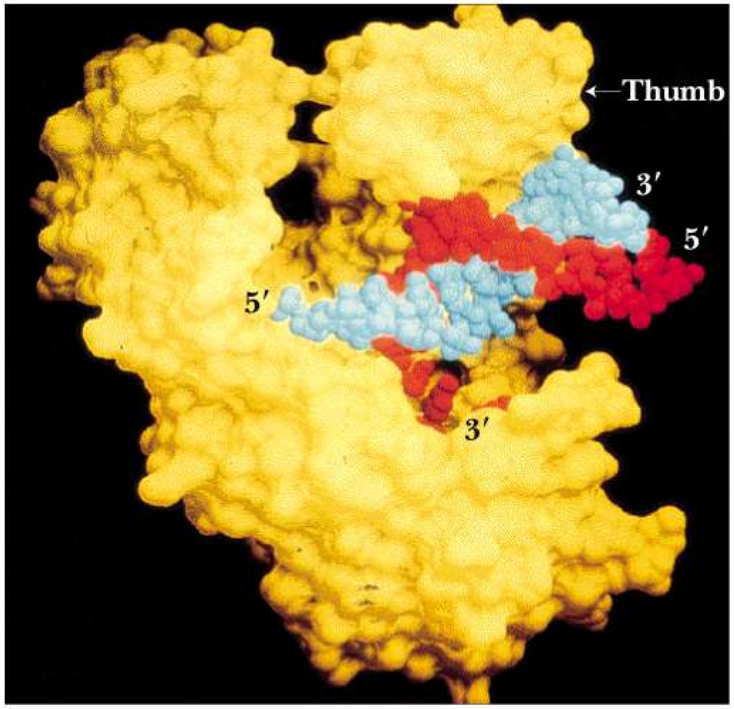
- DNAP I: functions in repair and replication
- DNAP II: functions in DNA repair (proven in 1999)
- DNAP III: principal DNA replication enzyme
- DNAP IV: functions in DNA repair (discovered in 1999)
- DNAP V: functions in DNA repair (discovered in 1999)

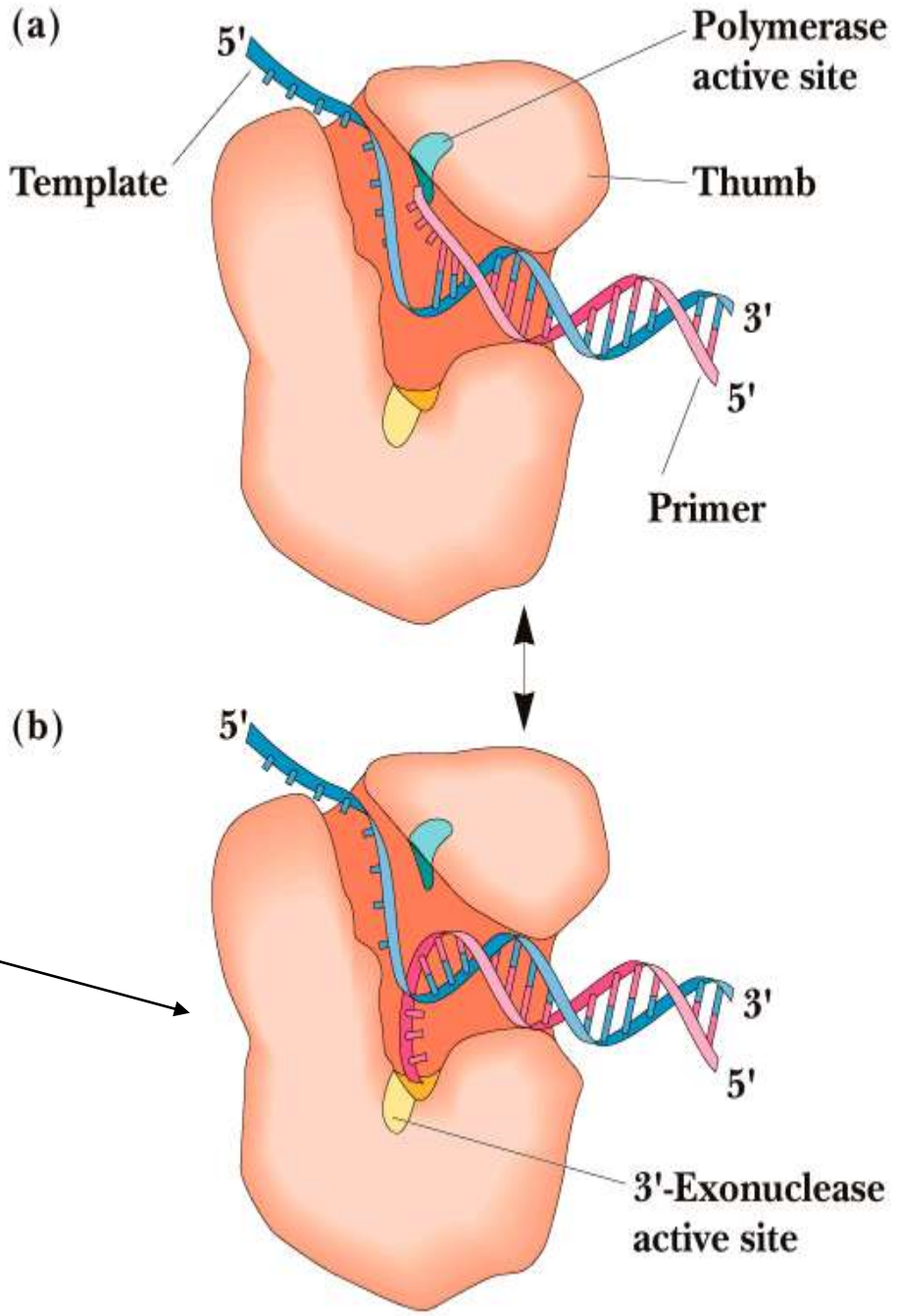
To date, a total of 14 different DNA polymerases have been reported in eukaryotes

DNA pol I

- First DNA pol discovered.
- Proteolysis yields 2 chains
  - Larger Chain (Klenow Fragment) 68 kd
    - C-terminal 2/3rd. 5'>>>3' polymerizing activity
    - N-terminal 1/3rd. 3'>>>5' exonuclease activity
  - Smaller chain: 5'>>>3 exonucleolytic activity
    - nt removal 5'>>>3'
    - Can remove >1 nt
    - Can remove deoxyribos or ribos

The structure of the Klenow fragment of DNAP I from E. coli



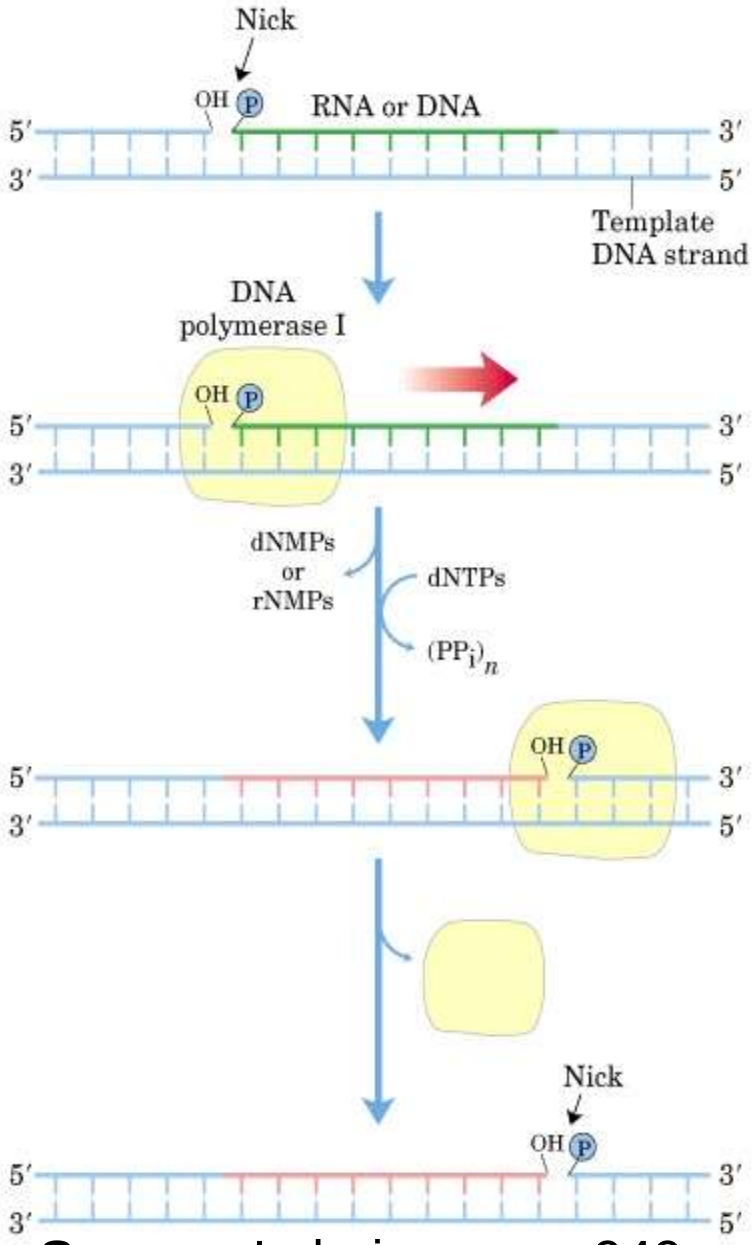


Nick Translation

Requires 5’-3’ activity of DNA pol I

Steps

1. At a nick (free 3’ OH) in the DNA the DNA pol I binds and digests nucleotides in a 5’-3’ direction
2. The DNA polymerase activity synthesizes a new DNA strand
3. A nick remains as the DNA pol I dissociates from the ds DNA.
4. The nick is closed via DNA ligase



Source: Lehninger pg. 940

DNA Polymerase I is great, but….

In 1969 John Cairns and Paula deLucia

-isolated a mutant bacterial strain with only 1% DNAP I activity (polA)

- mutant was super sensitive to UV radiation

- but otherwise the mutant was fine i.e. it could divide, so obviously it can replicate its DNA

Conclusion:

- DNAP I is NOT the principal replication enzyme in E. coli

Other clues….

- DNAP I is too slow (600 dNTPs added/minute)
- DNAP I is only moderately processive

  (processivity refers to the number of dNTPs added to a growing DNA chain before the enzyme dissociates from the template)

Conclusion:

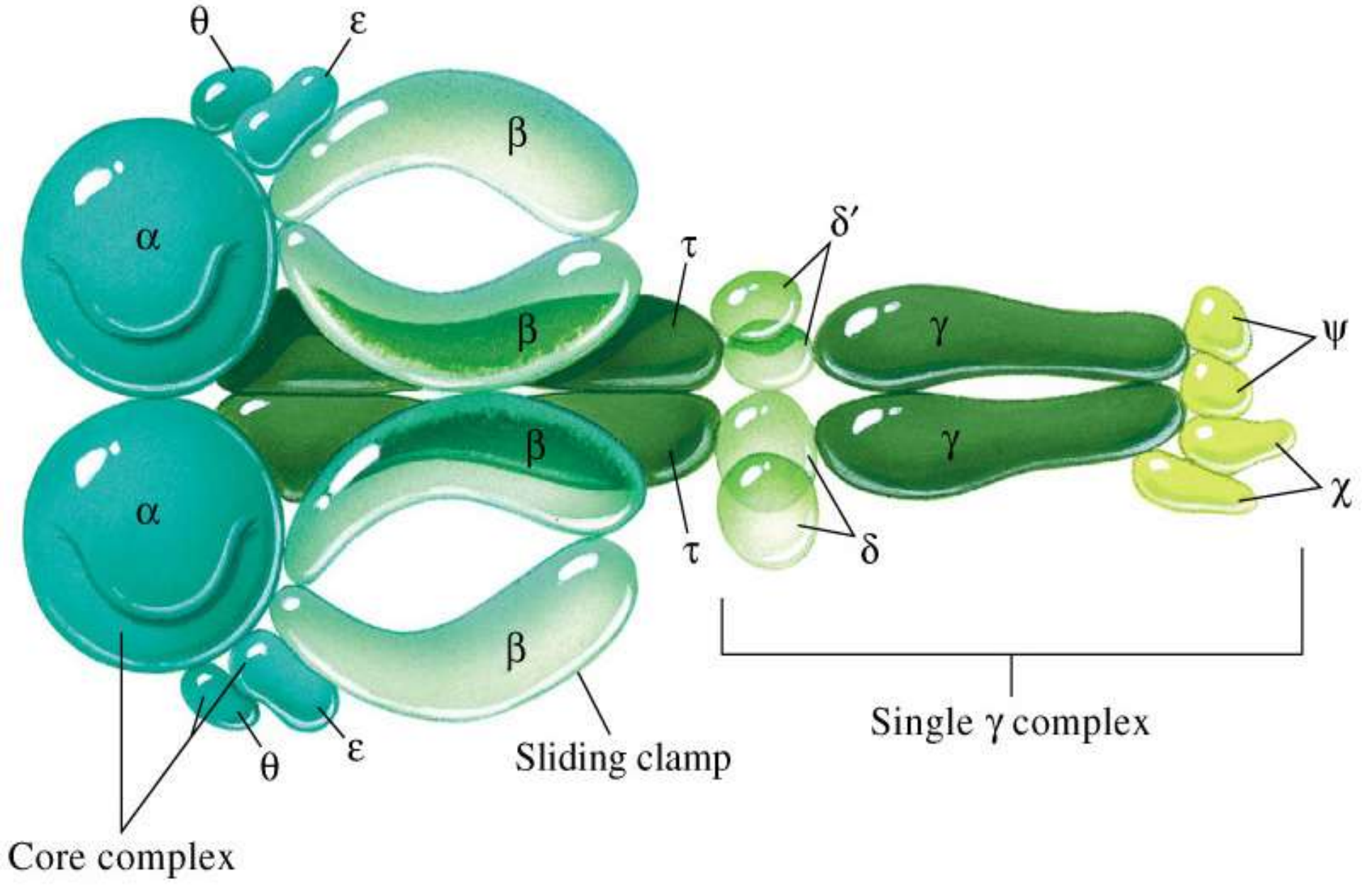
- There must be additional DNA polymerases.
- Biochemists purified them from the polA mutant

DNA Polymerase III

The major replicative polymerase in E. coli

- ~ 1,000 dNTPs added/sec
- It's highly processive: >500,000 dNTPs added before dissociating
- Accuracy:
  - 1 error in 10^7 dNTPs added,
  - with proofreading final error rate of 1 in 10^{10} overall.

DNA Polymerase III Holoenzyme (Replicase)



Components of *E. coli* DNA Polymerase III Holoenzyme.

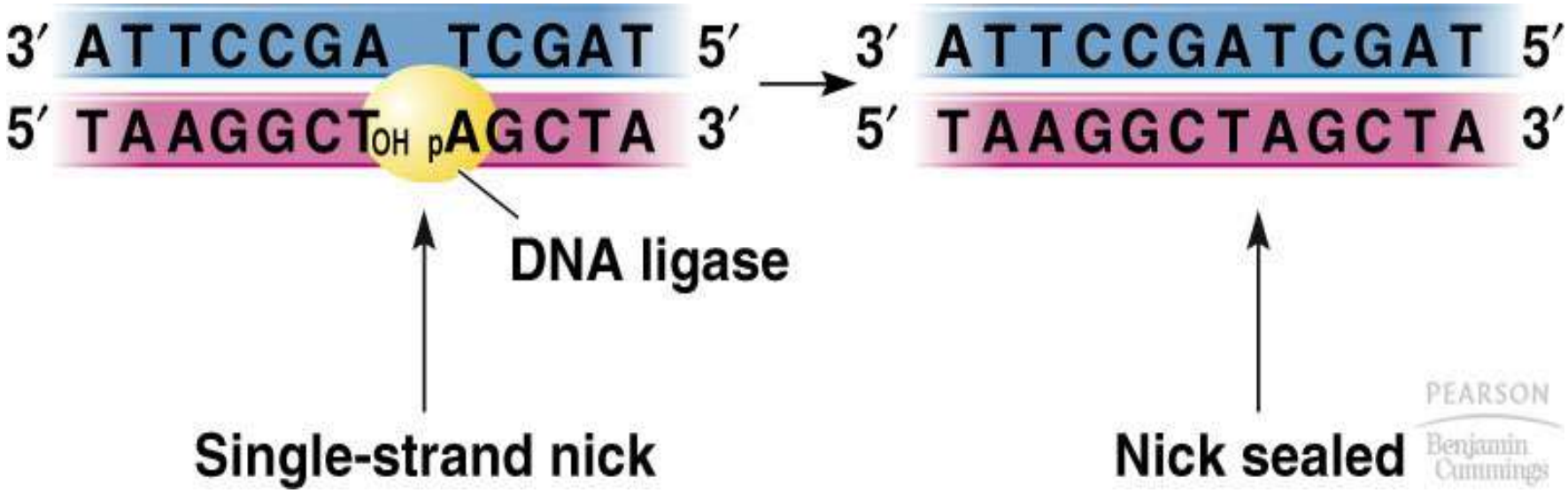
Subunit	Mass (kD)	Structural Gene
α^a	130	*polC (dnaE)*
ε^a	27.5	*dnaQ*
θ^a	10	*holE*
τ^b	71	*dnaX*[c]
γ^b	45.5	*dnaX*[c]
δ^b	35	*holA*
δ'^b	33	*holB*
χ^b	15	*holC*
ψ^b	12	*holD*
β	40.6	*dnaN*

[a]Components of the Pol III core.
[b]Components of the γ complex.
[c]The γ and τ subunits are encoded by the same gene sequence; the γ subunit comprises the N-terminal end of the τ subunit.

Sources: Kornberg, A. and Baker, T.A., *DNA Replication* (2nd ed.), p. 169, Freeman (1992); *and* Baker, T.A. and Wickner, S.H., *Annu. Rev. Genet.* **26,** 450 (1992).

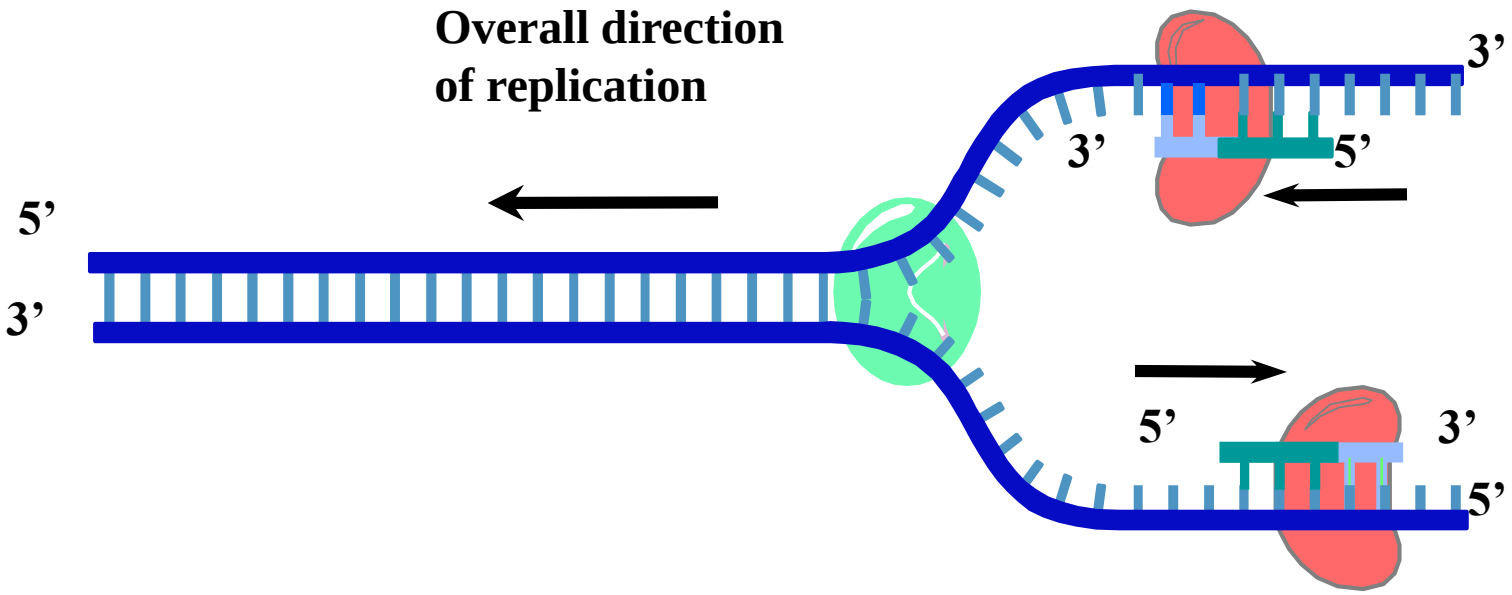
Activities of DNA Pol III

- ~900 kd
- Synthesizes **both** leading and lagging strand
- Can only extend from a primer (either RNA or DNA), not initiate
- 5'>>>3' polymerizing activity
- 3'>>>5' exonuclease activity
- NO 5'>>>3' exonuclease activity



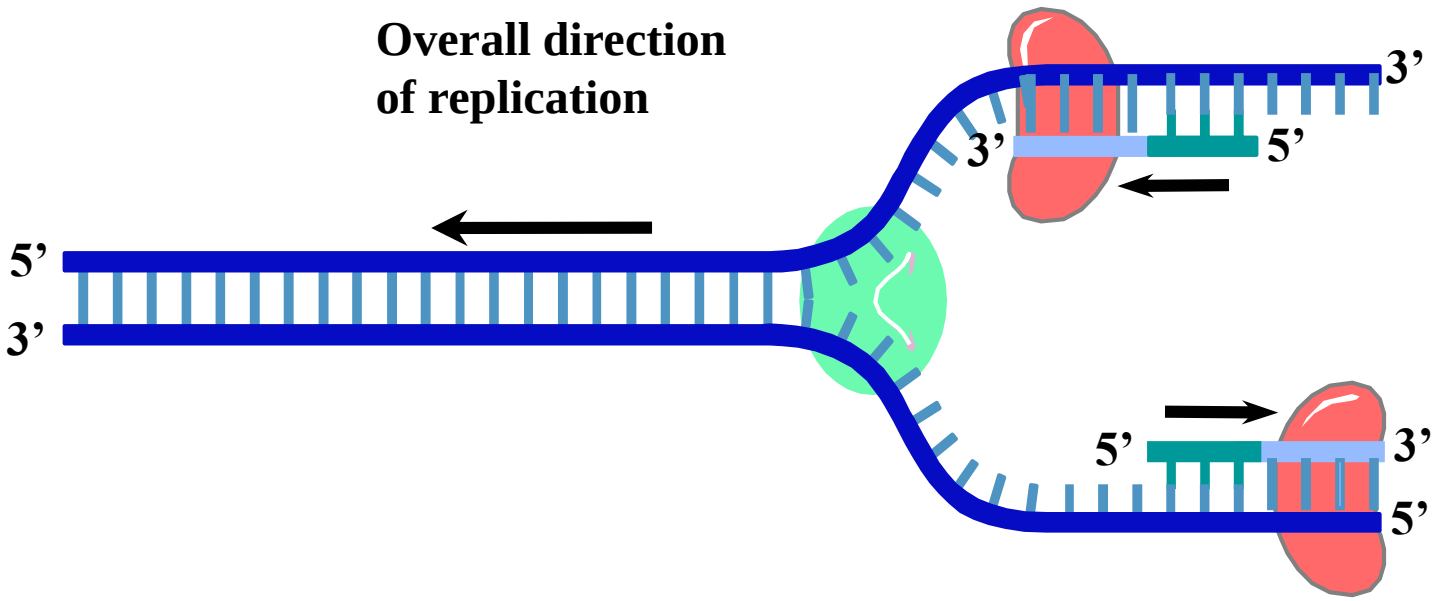
Action of DNA ligase in sealing the nick between adjacent DNA fragments (e.g., Okazaki fragments) to form a longer, covalently continuous chain. The DNA ligase catalyzes the formation of a phosphodiester bond between the 3’-OH and the 5’-phosphate groups on either side of a nick, sealing

Replication



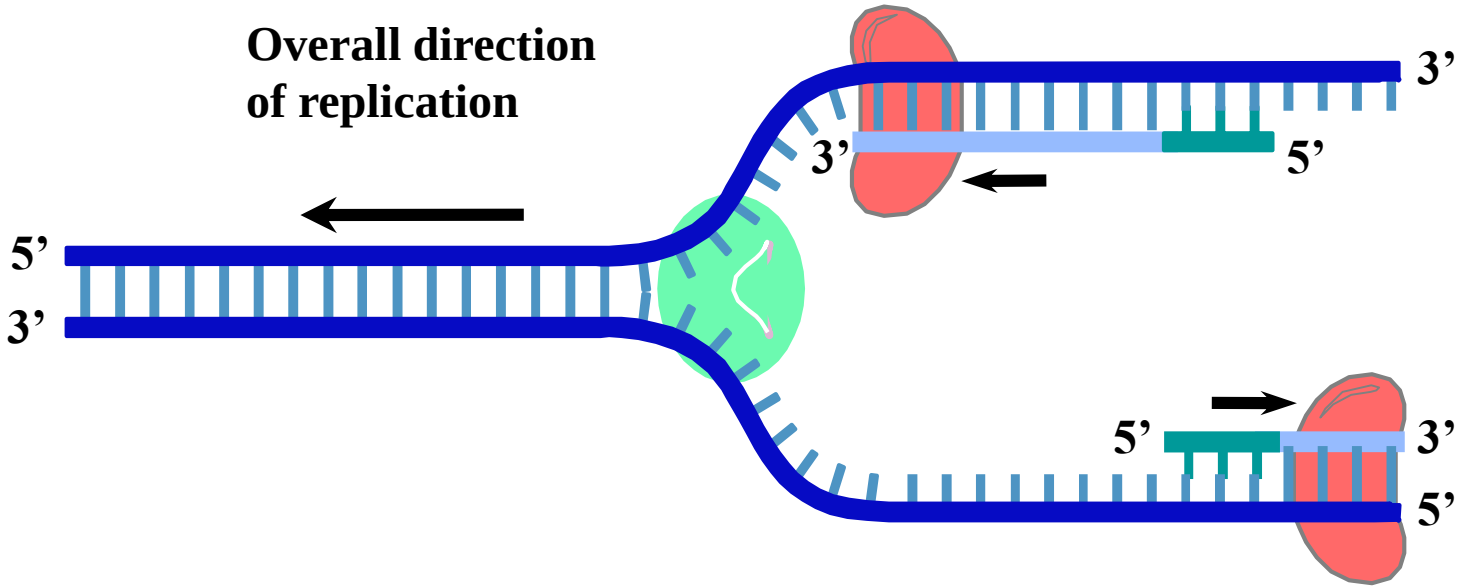
DNA polymerase III enzyme adds DNA nucleotides to the RNA primer.

Replication



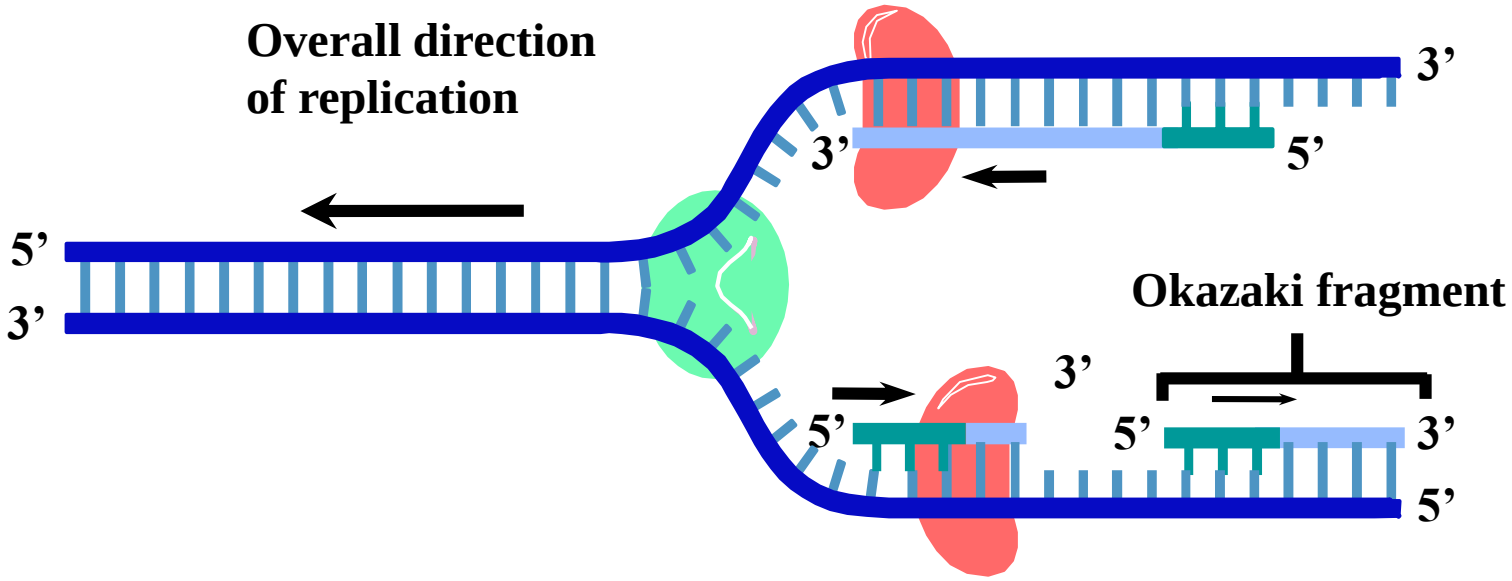
DNA polymerase proofreads bases added and replaces incorrect nucleotides.

Replication



Leading strand synthesis continues in a 5’ to 3’ direction.

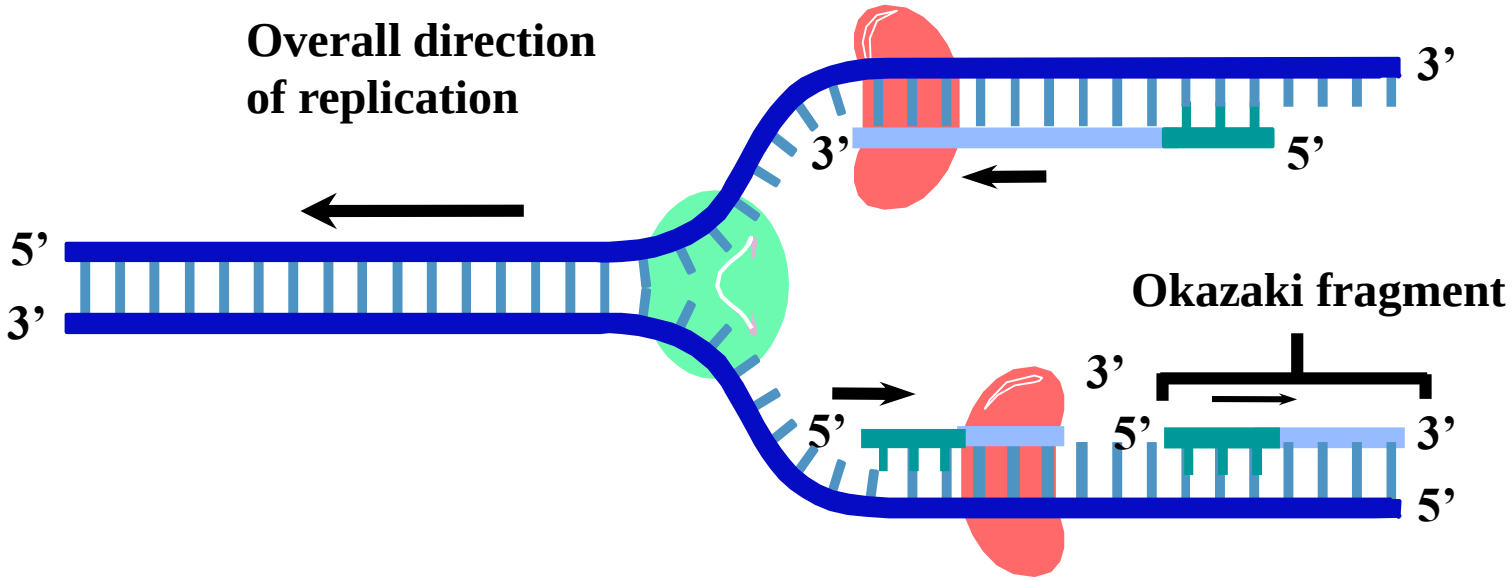
Replication



Leading strand synthesis continues in a 5’ to 3’ direction.

Discontinuous synthesis produces 5’ to 3’ DNA segments called Okazaki fragments.

Replication



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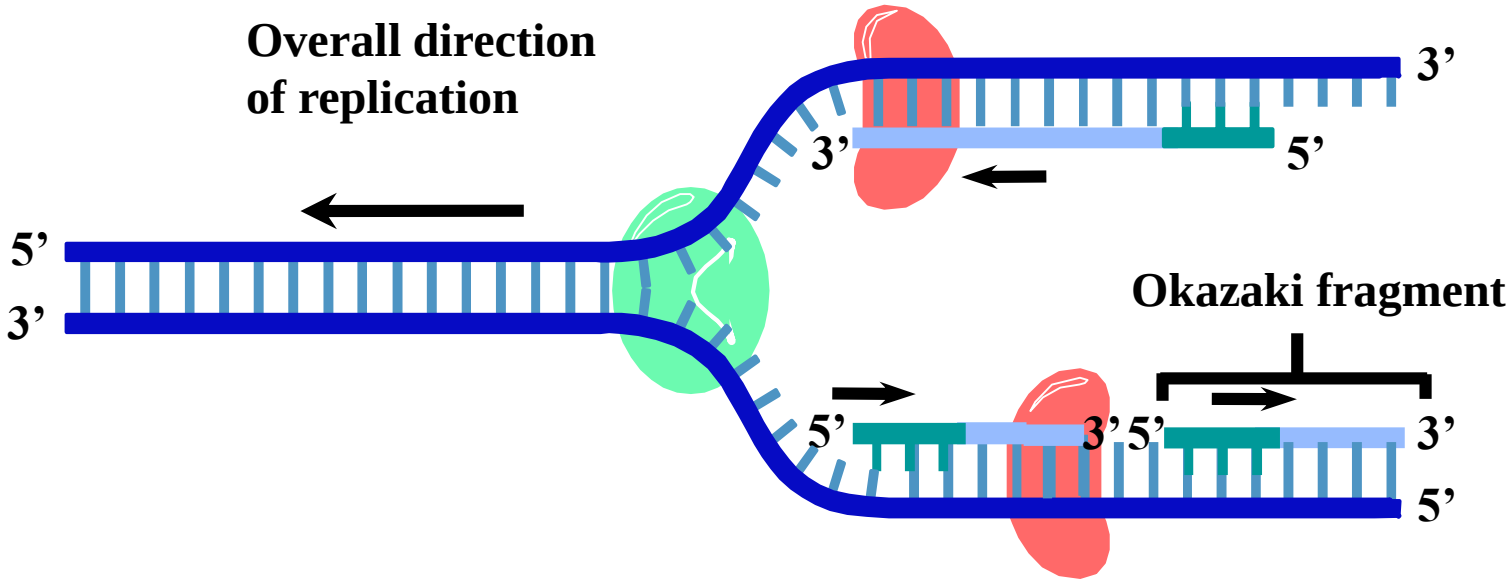

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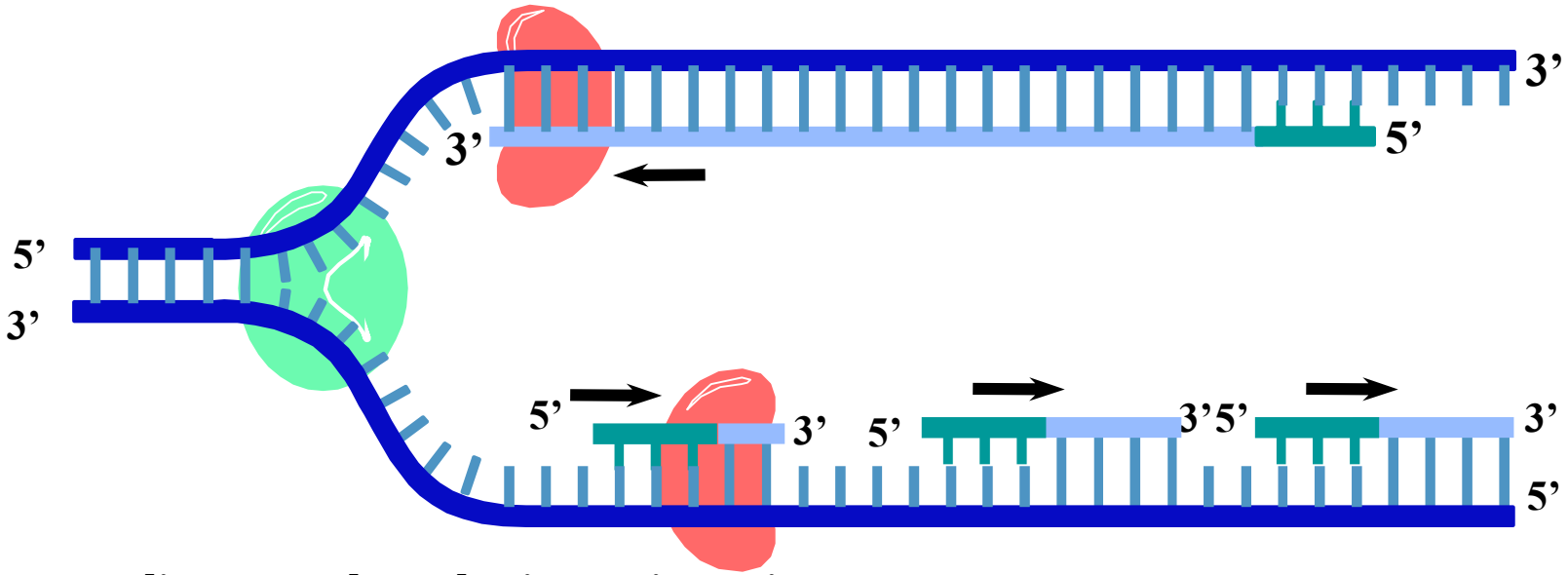

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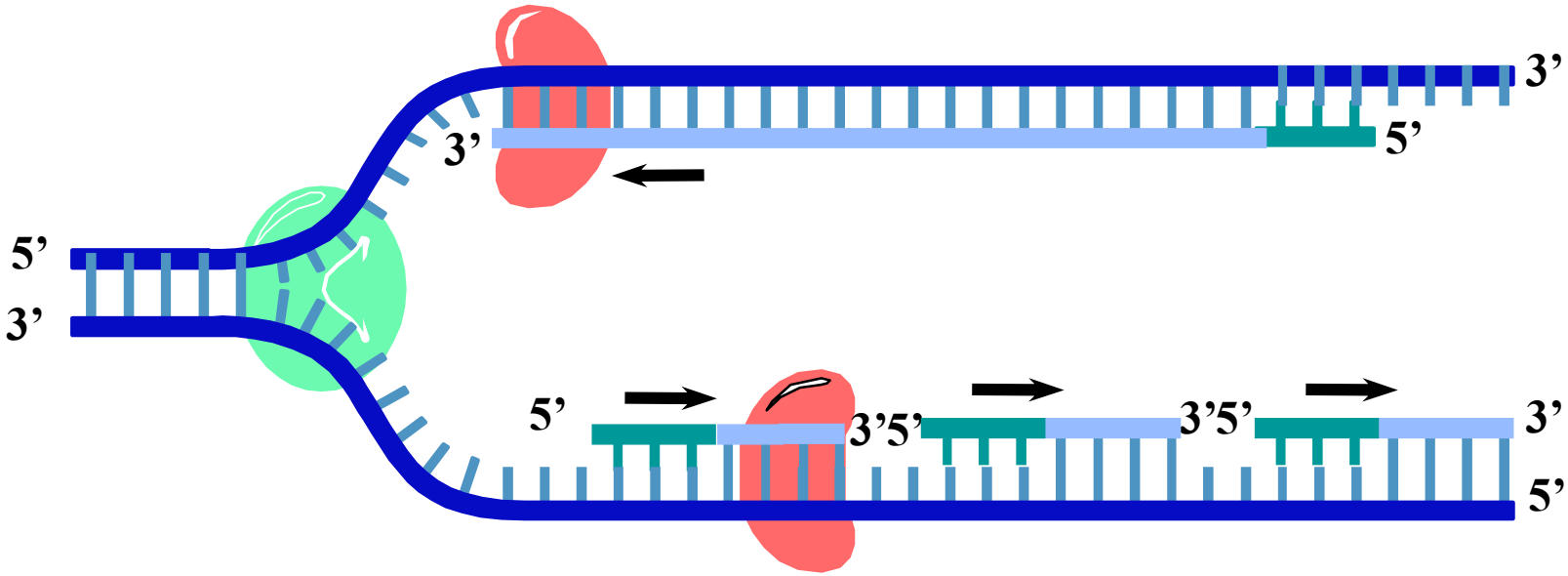
Discontinuous synthesis produces 5’ to 3’ DNA segments called Okazaki fragments.

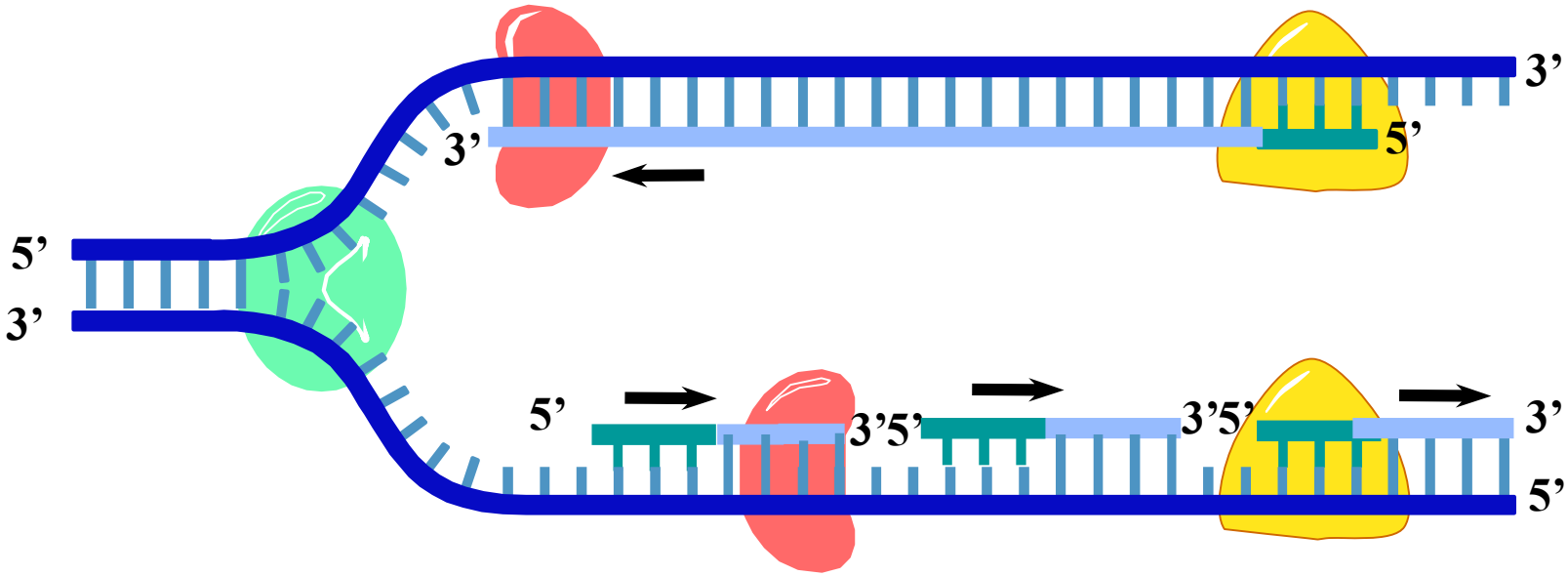
Replication



Leading strand synthesis continues in a 5’ to 3’ direction.

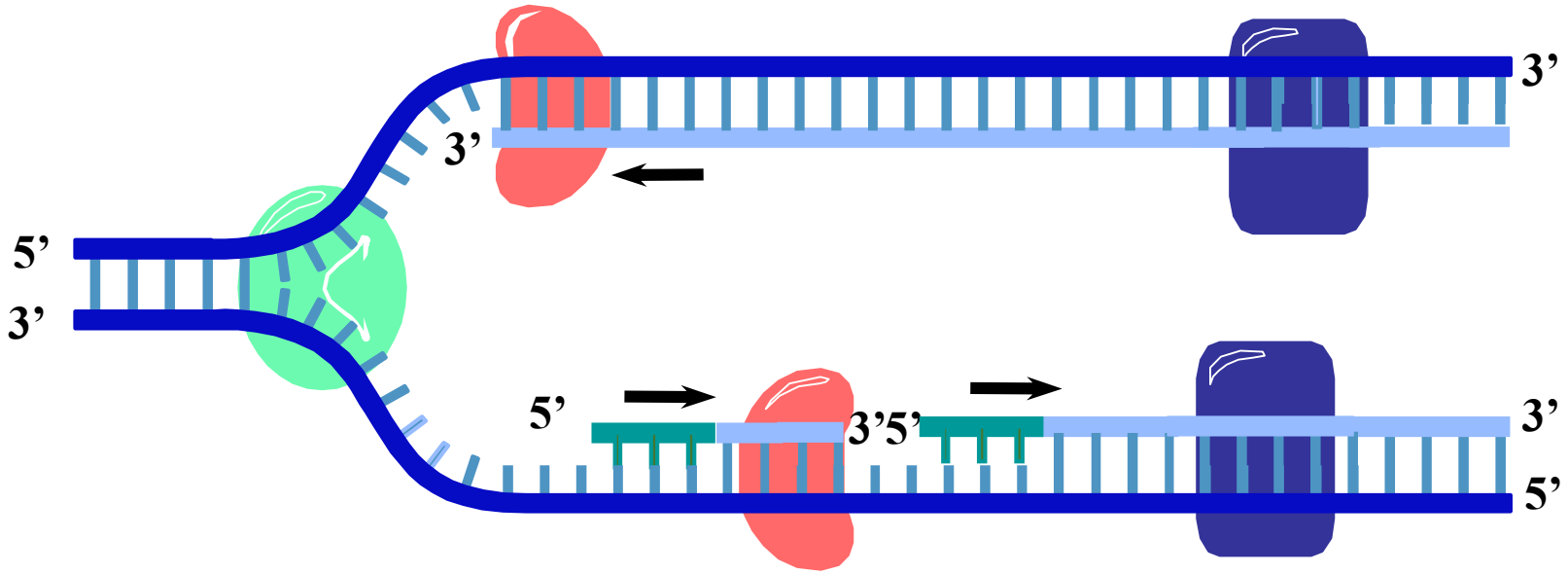
Discontinuous synthesis produces 5’ to 3’ DNA segments called Okazaki fragments.

Replication



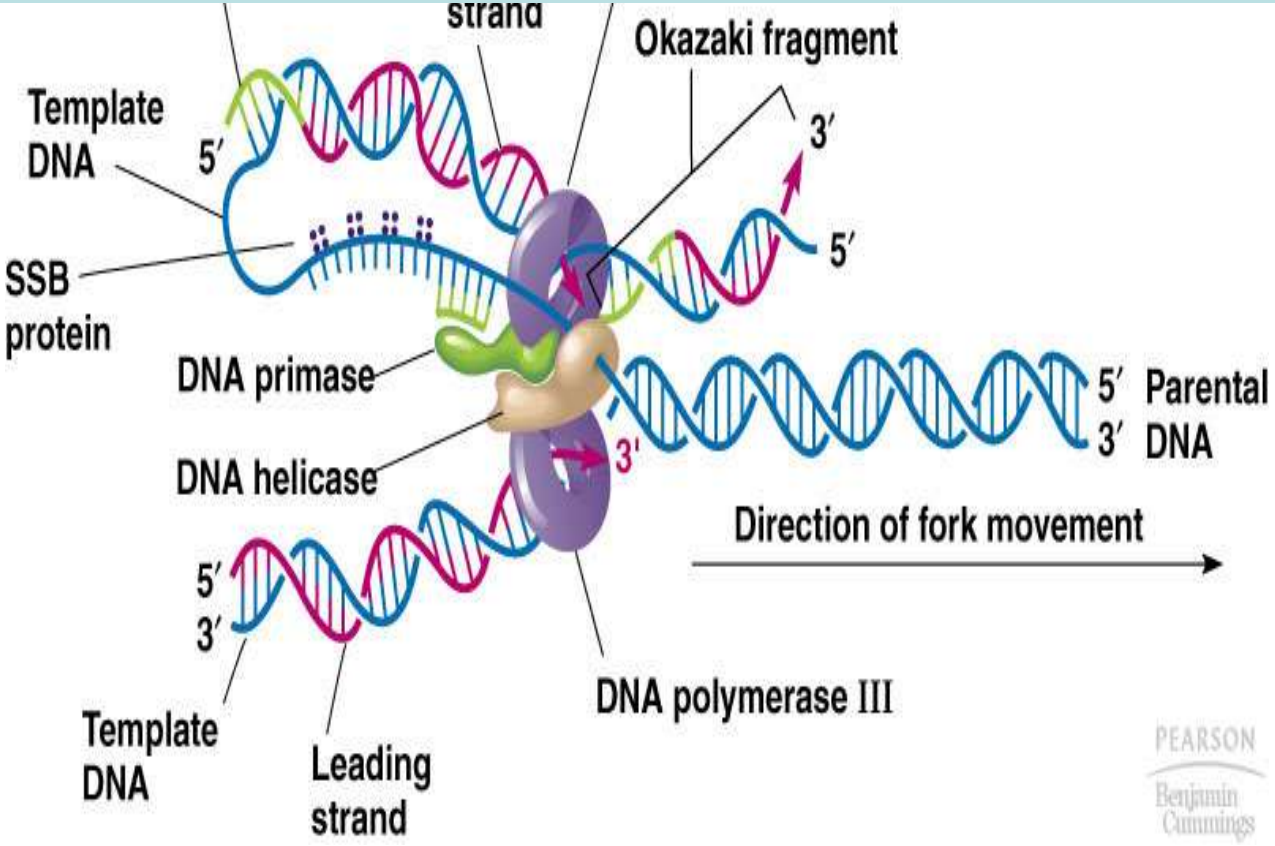
Exonuclease activity of DNA polymerase I removes RNA primers.

Replication

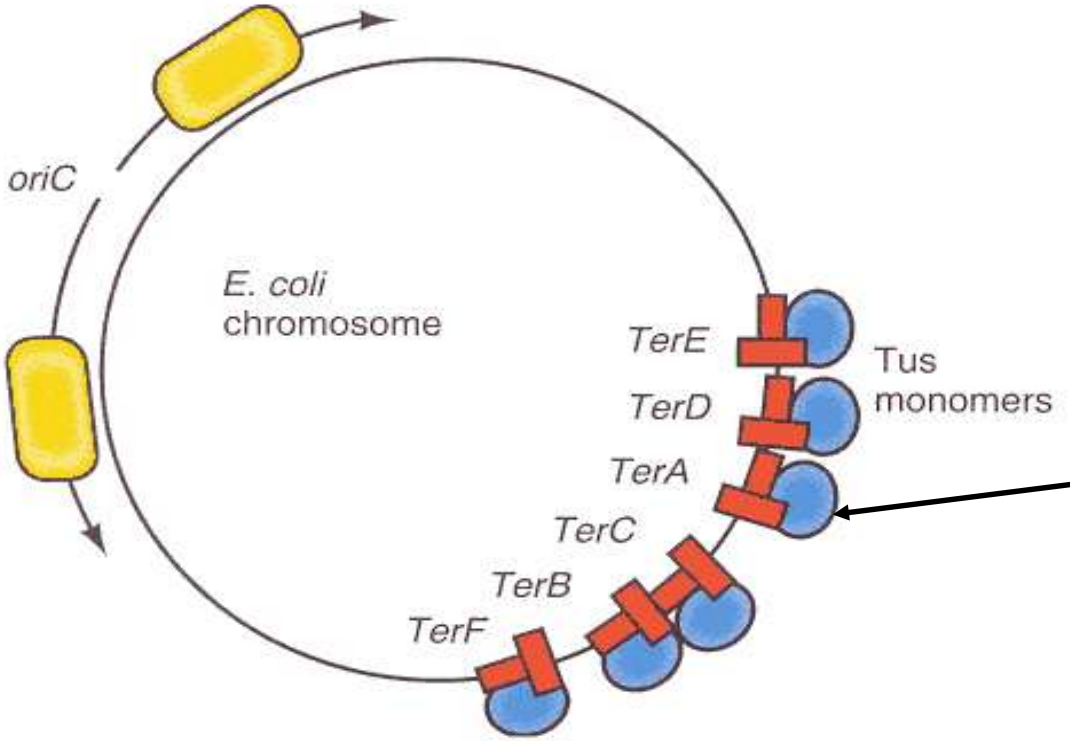


Polymerase activity of DNA polymerase I fills the gaps.
Ligase forms bonds between sugar-phosphate backbone.

Model for the replisome, the complex of key replication proteins, with the DNA at the replication fork. The DNA polymerase III on the lagging-strand template (top of figure) is just finishing the synthesis of an Okazaki fragment.



Termination of Replication



Tus Protein (309 aa) -arrests replication fork motion

- Occurs @ specific site opposite *ori c*
- ~350 kb
- Flanked by 6 nearly identical *non-palindromic**, 23 bp terminator (*ter*) sites
- * Significance?

Prokaryotic DNA Replication

Leading strand is synthesized continuously (in the same direction as the replication fork)

Lagging strand is synthesized discontinuously creating **Okazaki fragments**

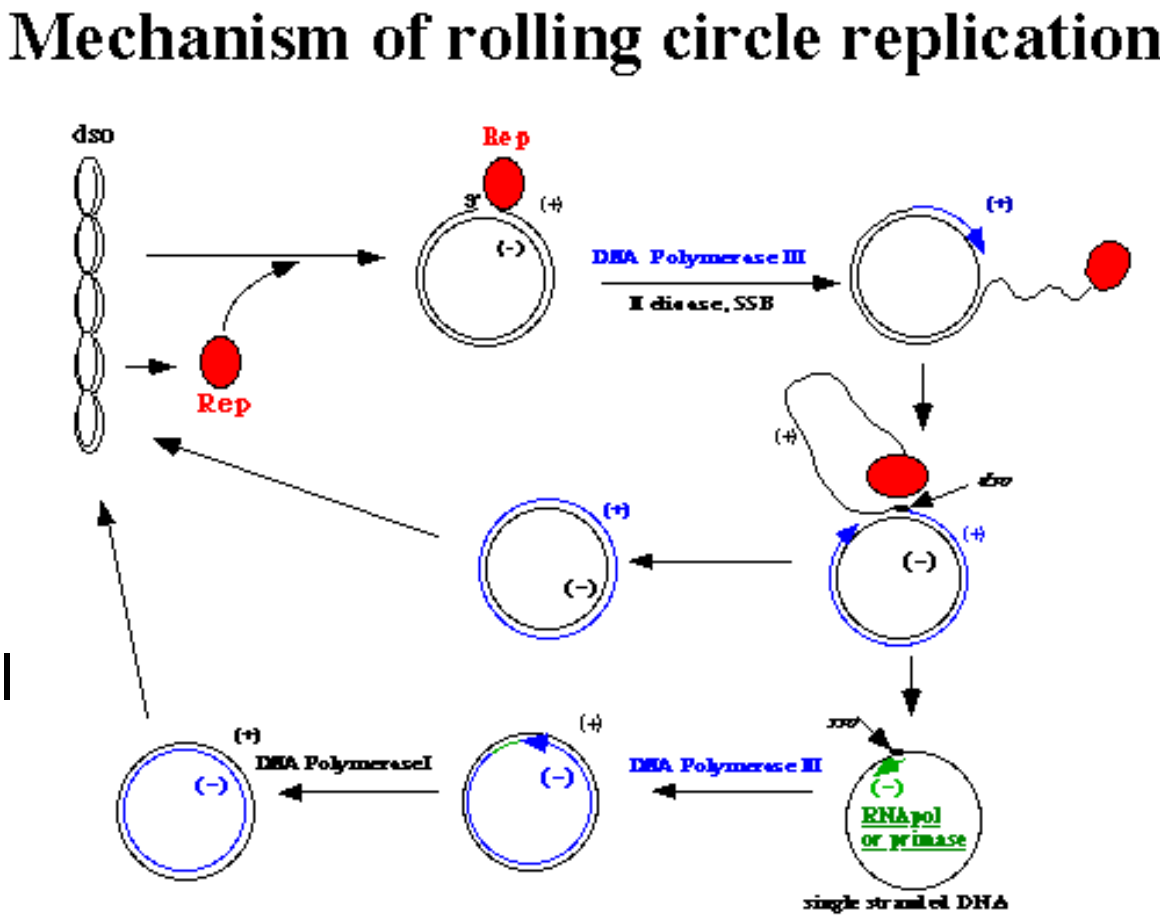
Types of DNA Replication

Based on the mode of directions

- **Uni-directional (Rolling Circle Replication)**
- **Bi-directional**

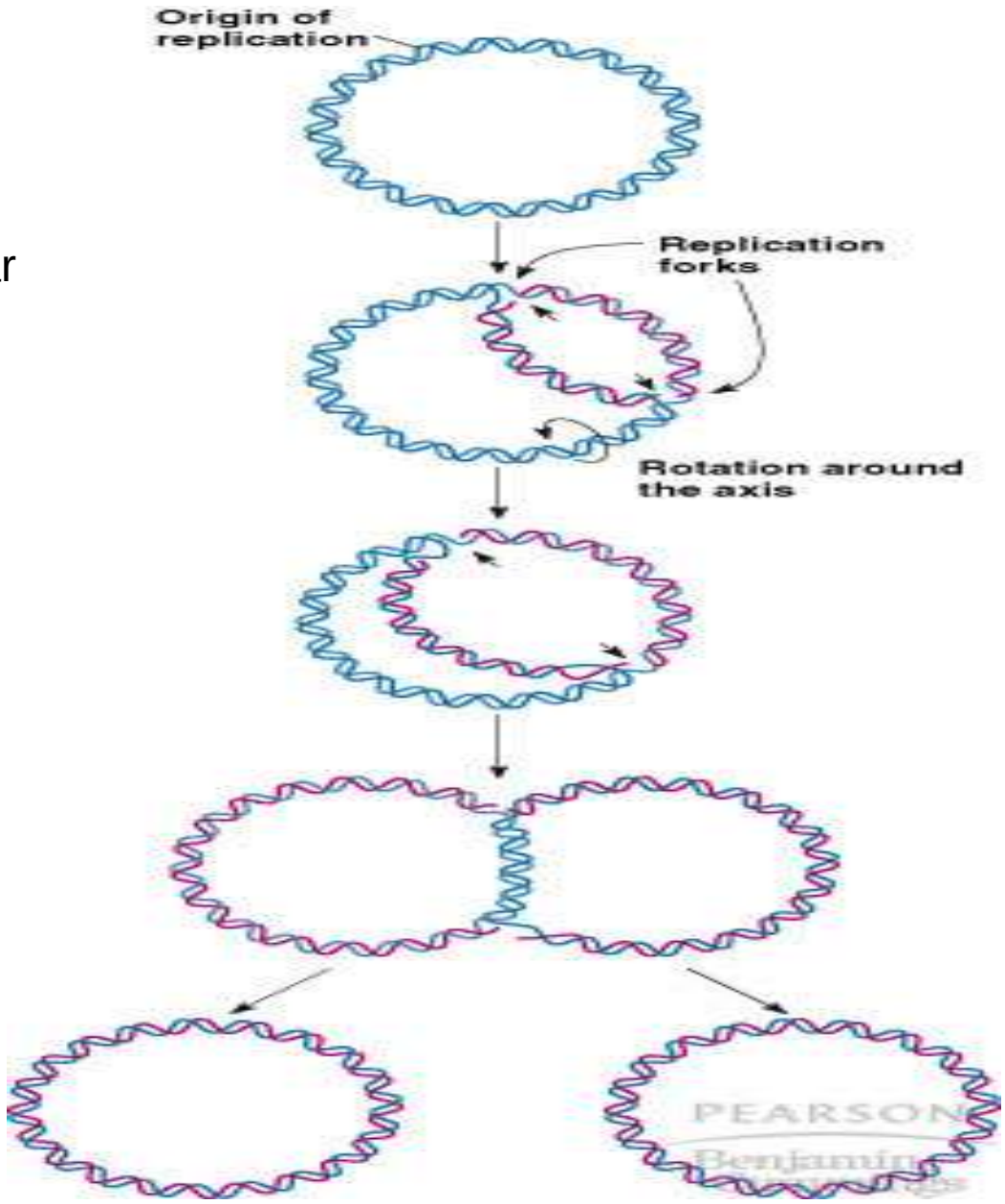
Rolling Circle Replication

- For the replication of
  - Circular DNA or RNA
  - Plasmids
  - Genomes of bacteriophages
  - Circular RNA genomes of viroids
  - Some eukaryotic viruses – HHV-6
- Uses
  - Used for amplifying DNA from very small amount



(Espinosa and al., 1995, FEMS Microbiology letters, 130: 111-120)

Bidirectional replication of circular DNA molecules.



Eukaryotic DNA Replication

The larger size and complex packaging of eukaryotic chromosomes means they must be replicated from multiple origins of replication.

The enzymes of eukaryotic DNA replication are more complex than those of prokaryotic cells.

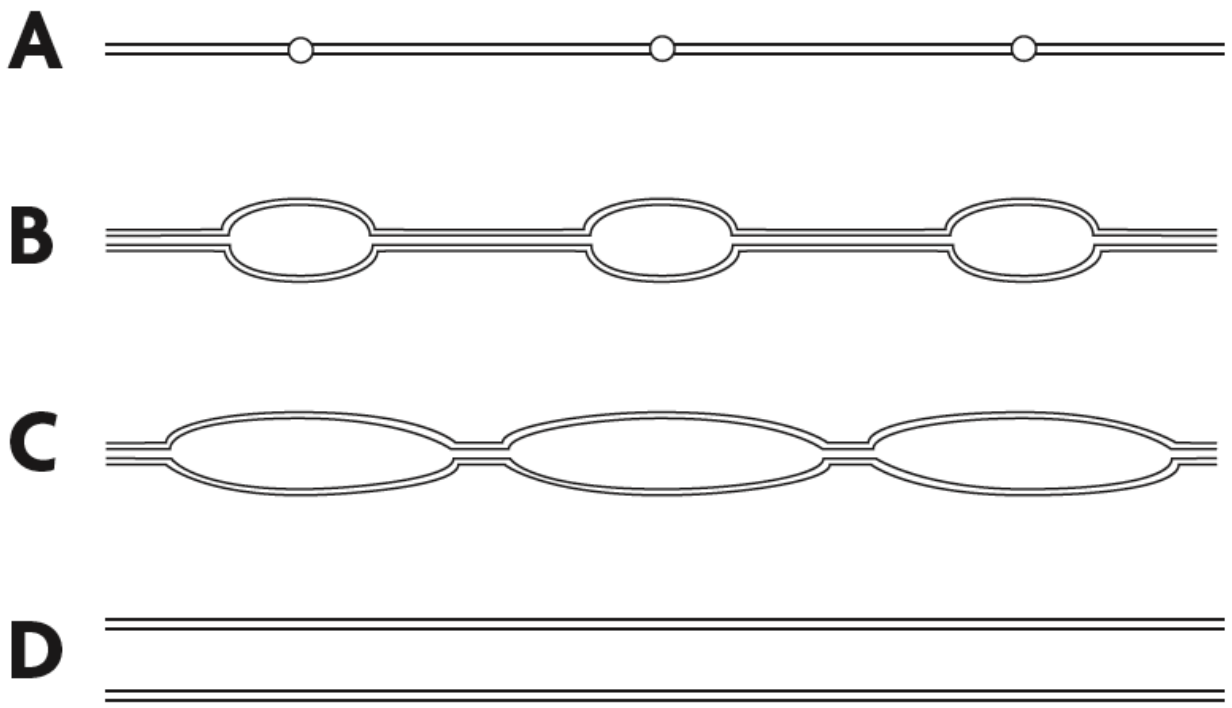
Eukaryotic DNA Replication

Synthesizing the ends of the chromosomes is difficult because of the lack of a primer.

With each round of DNA replication, the linear eukaryotic chromosome becomes shorter.

Replication is fast and accurate.

- DNA replication starts at many points in eukaryotic chromosomes.



There are many origins of replication in eukaryotic chromosomes.

- DNA polymerases can find and correct errors.

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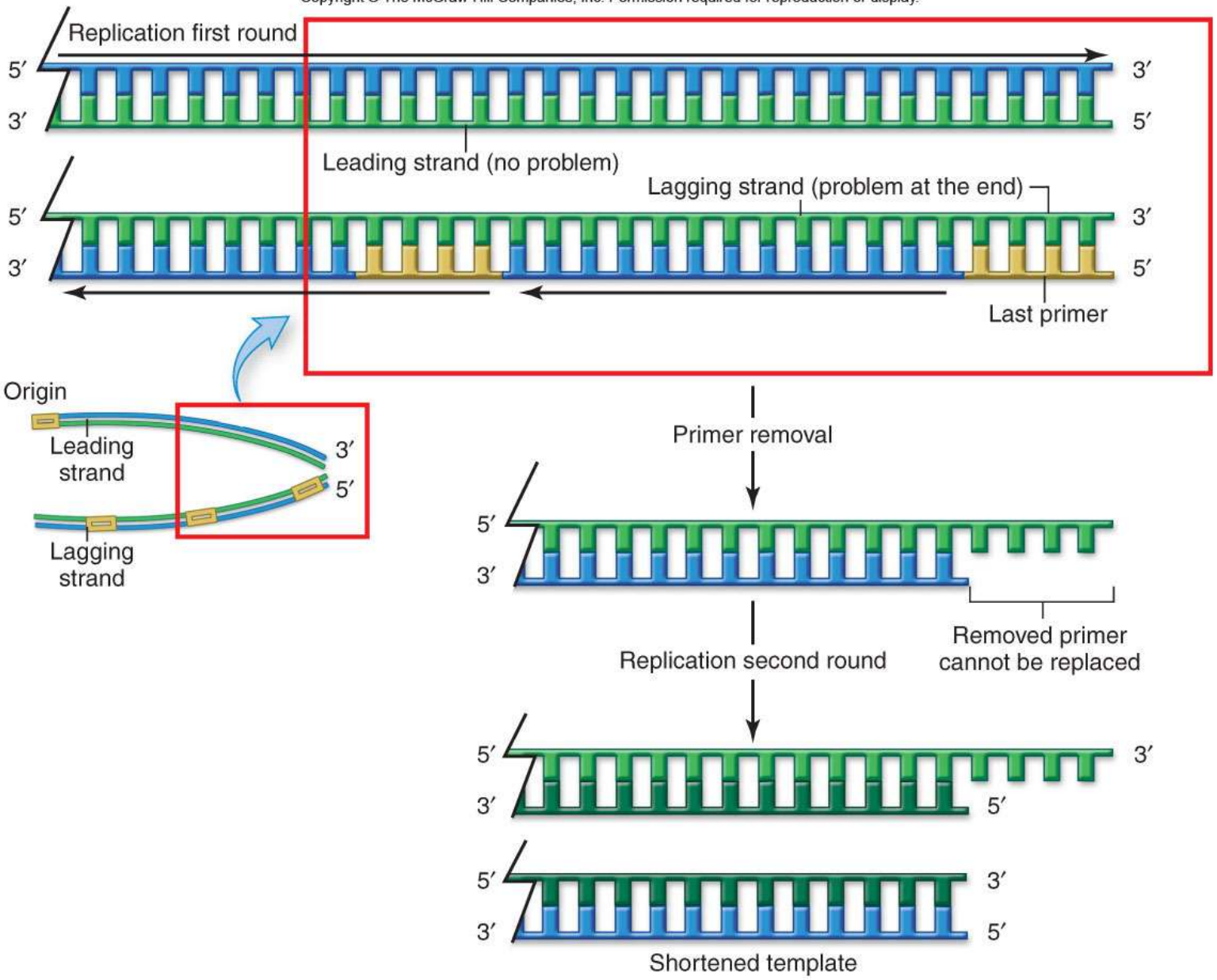

DNA Replication in Eukaryotes

Eukaryotes have at least 15 DNA Polymerases:

- **Pol α** : act as a primase (synthesizing an RNA primer), elongates the primer
- **Pol β :** repairs DNA, (excision repair and gap-filling).
- **Pol γ**: Replicates and repairs mitochondrial DNA and has proofreading 3' → 5' exonuclease activity.
- **Pol δ**: Highly processive and has proofreading 3' → 5' exonuclease activity, reposible for replication of lagging strand.
- **Pol ε**: Highly processive and has proofreading 3' → 5' exonuclease activity, reponsible for replication of leading strand.
- **η**, **ι**, **κ**, **Rev1** and **Pol ζ** are involved in the bypass of DNA damage.
- **θ**, **λ**, **φ**, **σ**, and **μ are** not as well characterized:
- There are also others, but the nomenclature has become quite jumbled.

DNA Replication in Eukaryotes

- the polymerases that deal with the elongation are **Pol α, Pol ε,Polδ**.

- **Pol α** : forms a complex to act as a primase (synthesizing an RNA primer), and then elongates that primer with DNA nucleotides.

- After around 20 nucleotides elongation by Pol α is taken over by Pol ε (on the leading strand) and δ (on the lagging strand).

- Other enzymes are responsible for primer remover in Eukaryotes as none of their polymerases have 5′→3′ exonuclease activity

Eukaryotic DNA Replication

Telomeres – repeated DNA sequence on the ends of eukaryotic chromosomes

- produced by **telomerase**

Telomerase contains an RNA region that is used as a template

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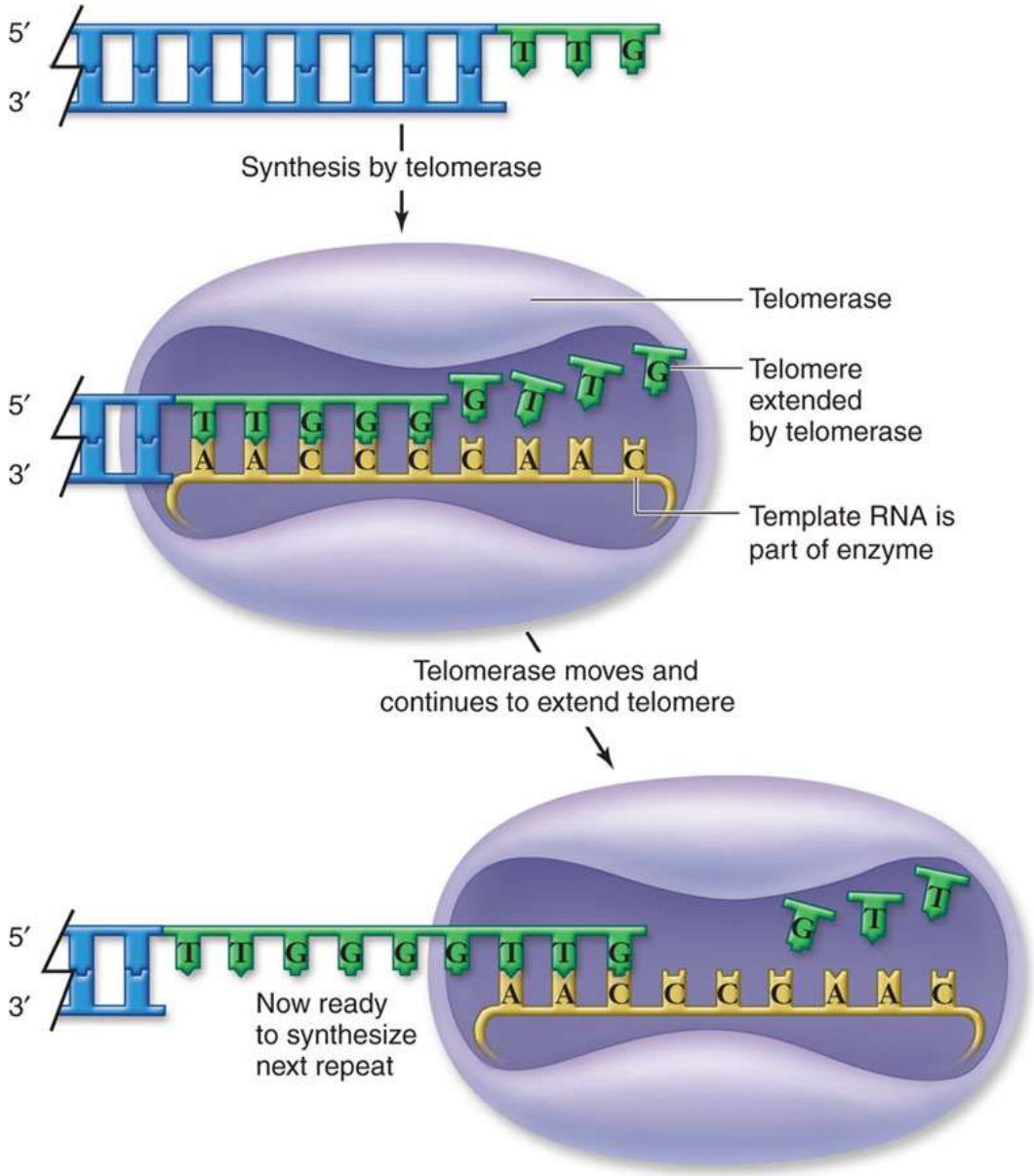
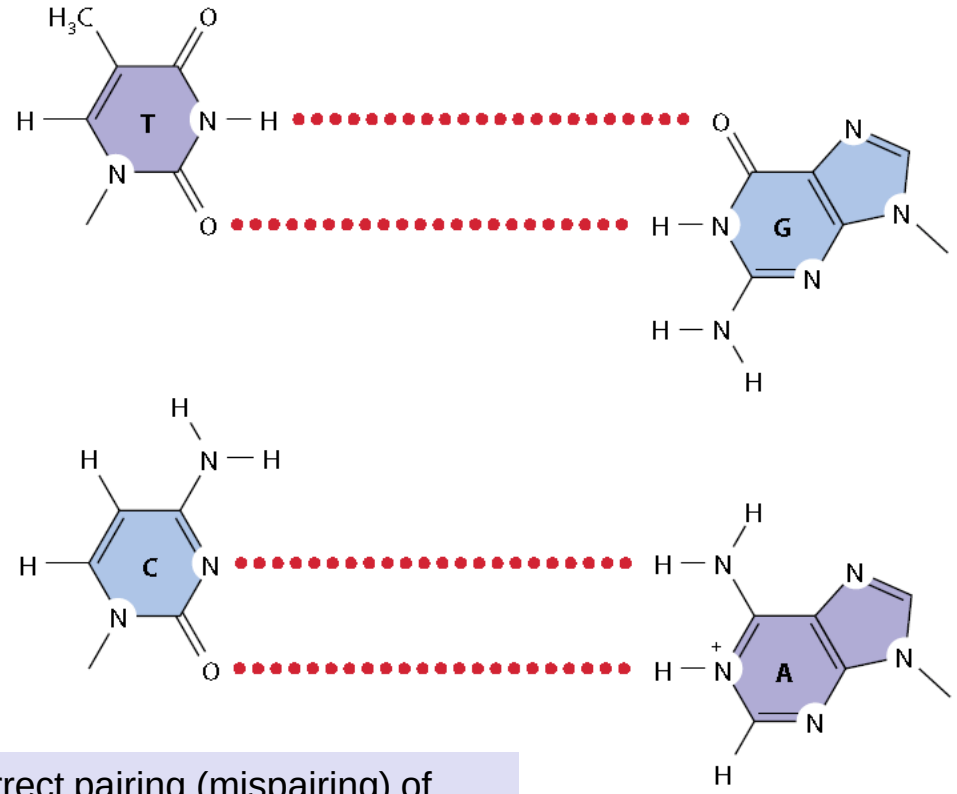


Errors During DNA Replication

A human cell can copy its entire DNA in a few hours, with an error rate of about one per 1 billion nucleotide pairs.

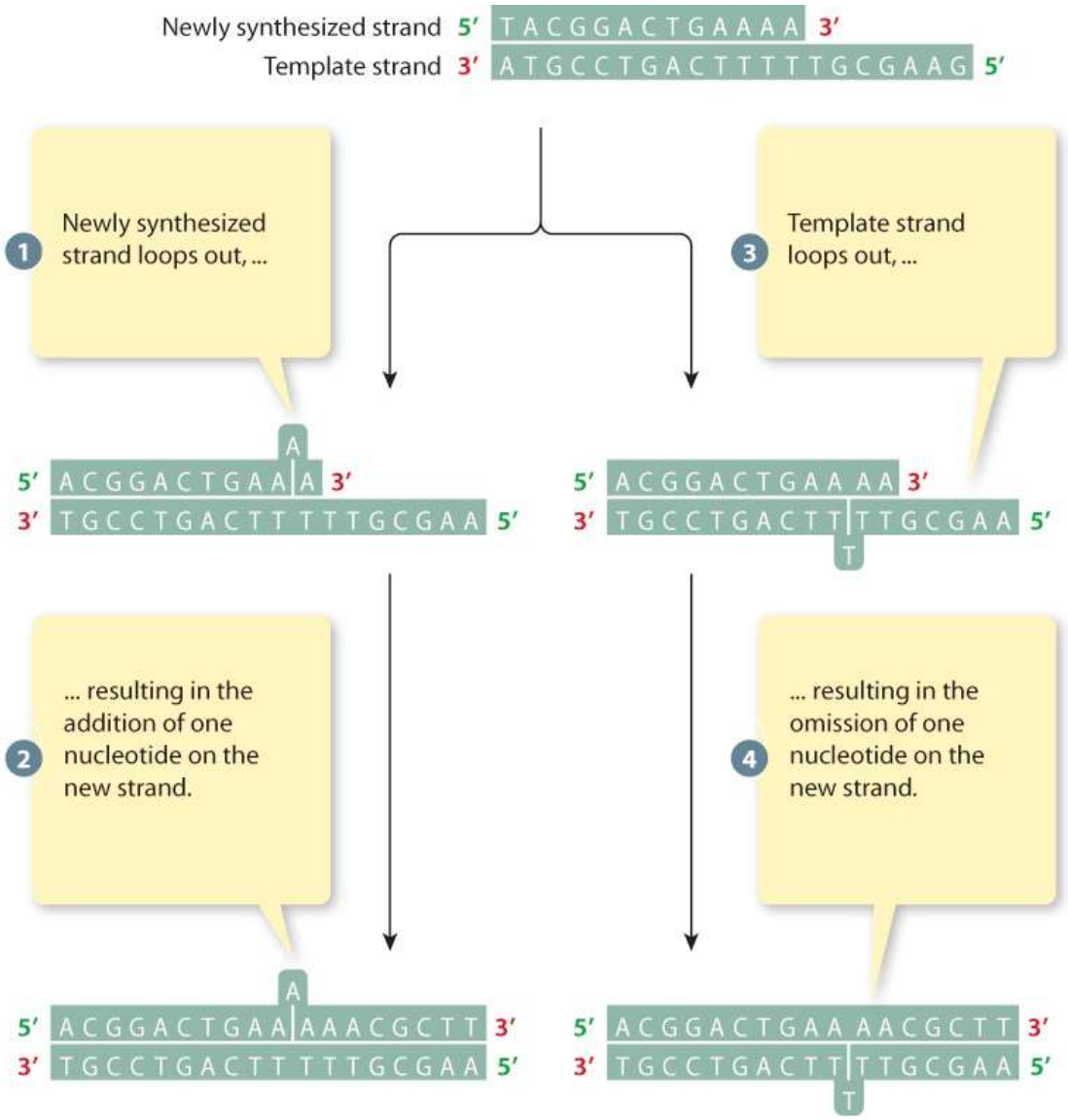
Errors naturally occur during replication. Mispairing of bases and strand slippage are two types of errors that cause either additions or omissions of nucleotides.



Incorrect pairing (mispairing) of bases is thought to occur as a result of flexibility in DNA structure.

Errors During DNA Replication

Strand slippage during DNA replication can cause the addition or omission of nucleotides in newly synthesized strands, which represent errors.



Correcting Errors During DNA Replication

DNA polymerase I and **II** have proofreading abilities. These enzymes recognize and correct errors in newly synthesized strands of DNA. This method repairs about 99% of the mismatch errors that occur during replication.

Mismatch repair is done by a group of proteins that can recognize and repair deformities in newly synthesized DNA that feature the mispairing of bases.

Errors that remain after DNA polymerase proofreading or mismatch repair are considered mutations once cell division occurs.

Comparing DNA Replication in Eukaryotes and Prokaryotes

Similarities	Differences
• the requirement of an origin of replication • elongation in the 5′ to 3′ direction • continuous synthesis of the leading strand and discontinuous synthesis of the lagging strand • the use of a primer for the synthesis of Okazaki fragments • the use of DNA polymerase enzymes	• the rate of replication is faster in prokaryotes • DNA polymerase enzymes are different in structure and number • the small, circular chromosome of a prokaryote contains a single origin of replication whereas the larger, linear eukaryotic chromosome contains thousands • linear chromosomes contain telomeres (synthesized by telomerases), which are highly repetitive sequences on the ends of chromosomes that ensure important genetic material is not lost during lagging strand replication

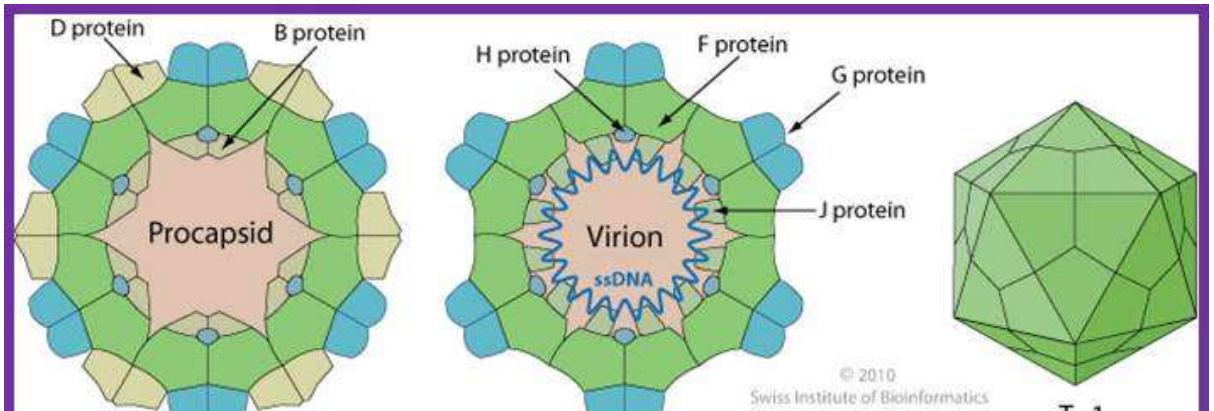
Bacteriophage ϕX174 DNA Replication

φX174 (phiX174) is a virus that infects the bacterium *E. coli*.

First DNA-based genome (5386 bp) to be sequenced by Fred Sanger (1977).

The genome of ΦX174 was the first to be completely assembled *in vitro* from synthesized oligonucleotides (JCV, 2003).

Non-enveloped, round, icosahedral symmetry (T=1), about 30 nm in diameter. The capsid consists of 12 pentagonal trumpet-shaped pentamers. The virion is composed of 60 copies each of the F, G, and J proteins, and 12 copies of the H protein. There are 12 spikes which are each composed of 5 G and one H proteins.



Genetic Map of ϕX 174

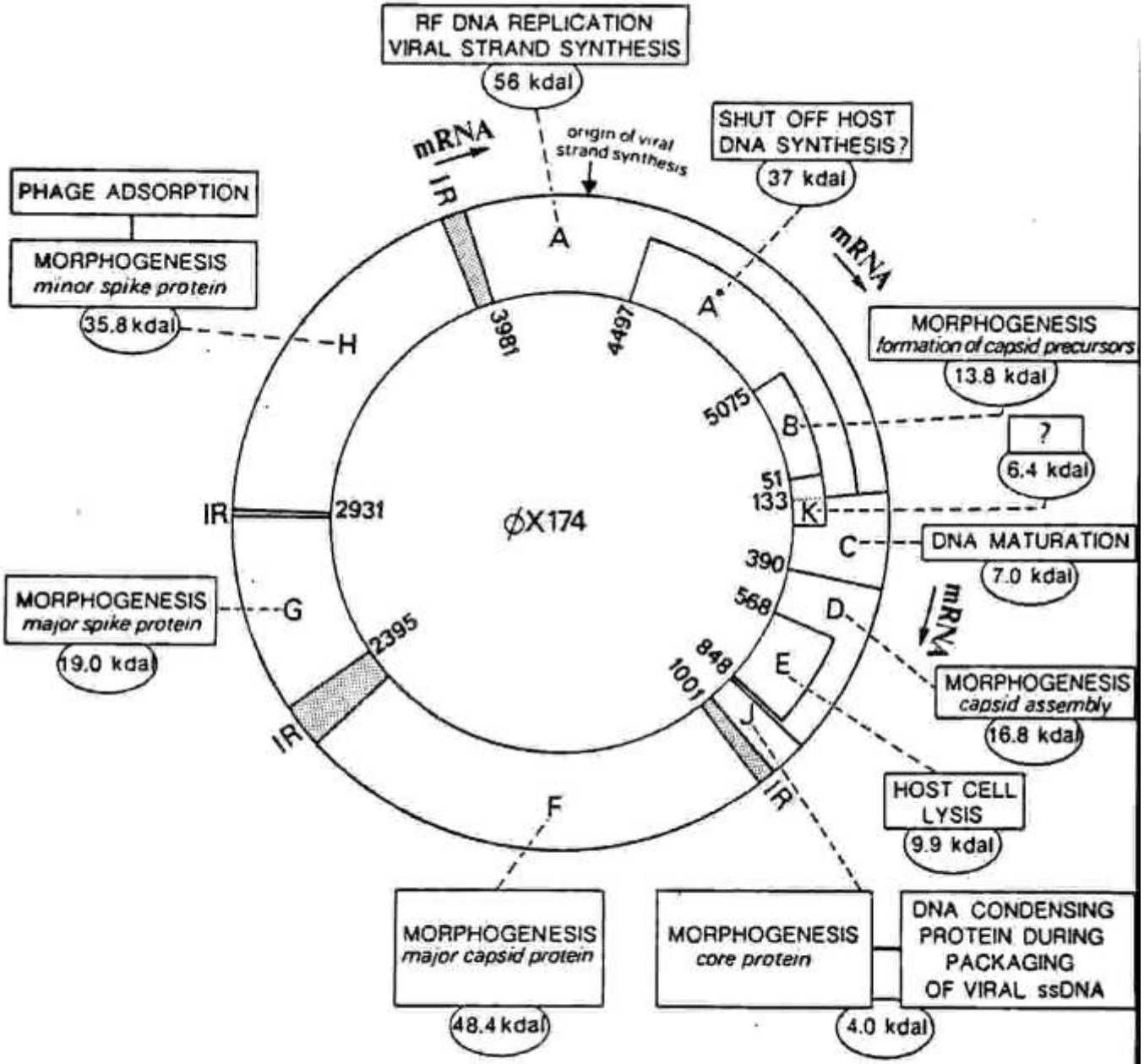


FIGURE S14-2

The SS(c) – RFI Reaction

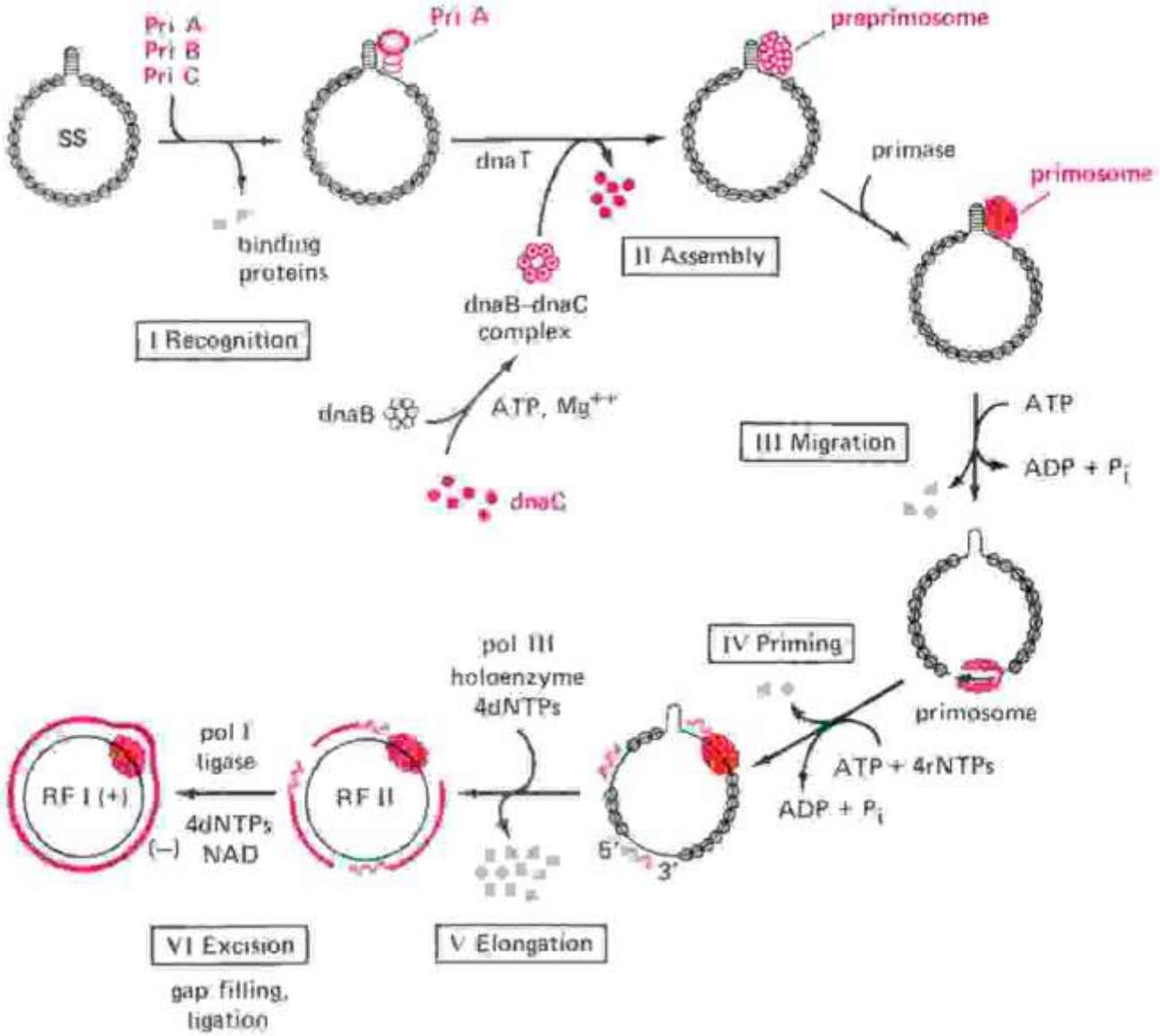


Figure 8-8
Scheme for assembly and migration of the primosome and the stepwise displacement of SSB in the ϕX SS → RF reaction.

The RF to RF Pathway

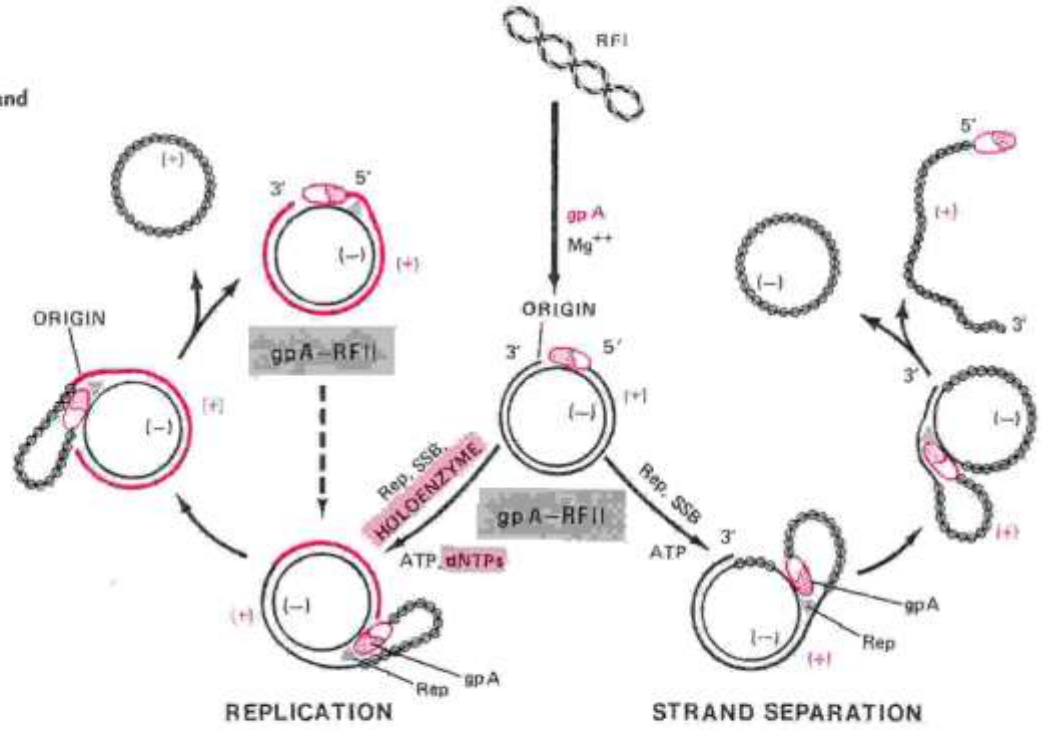


Figure 8-13
Scheme for gpA action, illustrating its multiple functions. The looped rolling-circle intermediate form is used in strand separation, uncoupled from replication, as well as in the synthesis of viral (+) strands. Rep = the Rep helicase.

The Origin of Replication of Icosahedral Phages ϕX174 and G4

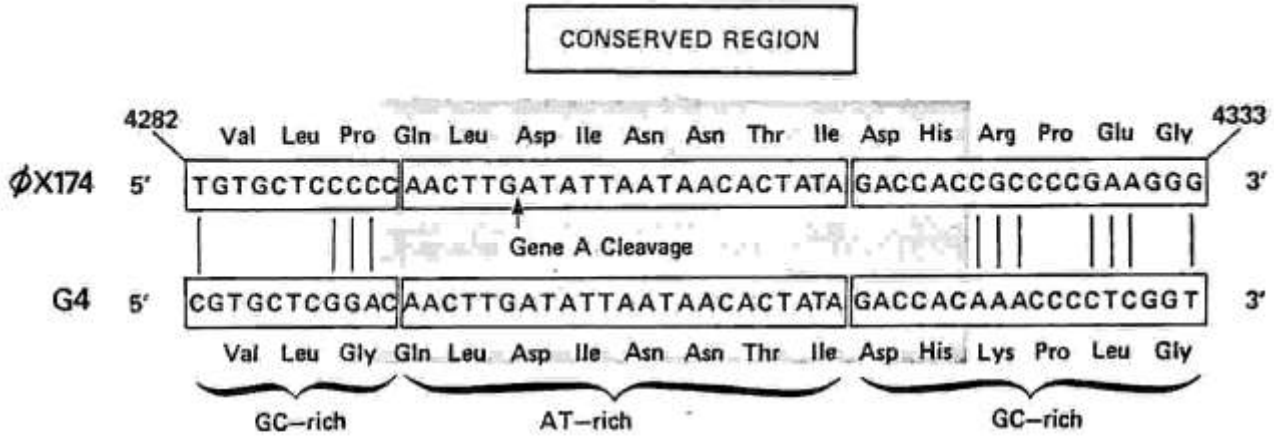


FIGURE 13-8
Nucleotide sequence at origin of viral strand in replicative form of ϕX174 and G4. Covalent extension from point of cleavage by gene A protein (residues 4297–4298 on PstI map) starts synthesis of viral strand in the direction of the arrow. The AT-rich region of ϕX174 is completely conserved in G4 and neighboring GC-rich regions are homologous. Differences in base sequence between ϕX174 and G4 are marked with lines. [Adapted from Fiddes, J. C., Barrell, B. G., and Godson, G. N. (1978) *PNAS 75*, 1081.]

The gpA Catalyzed Cleavage and Ligation Reaction

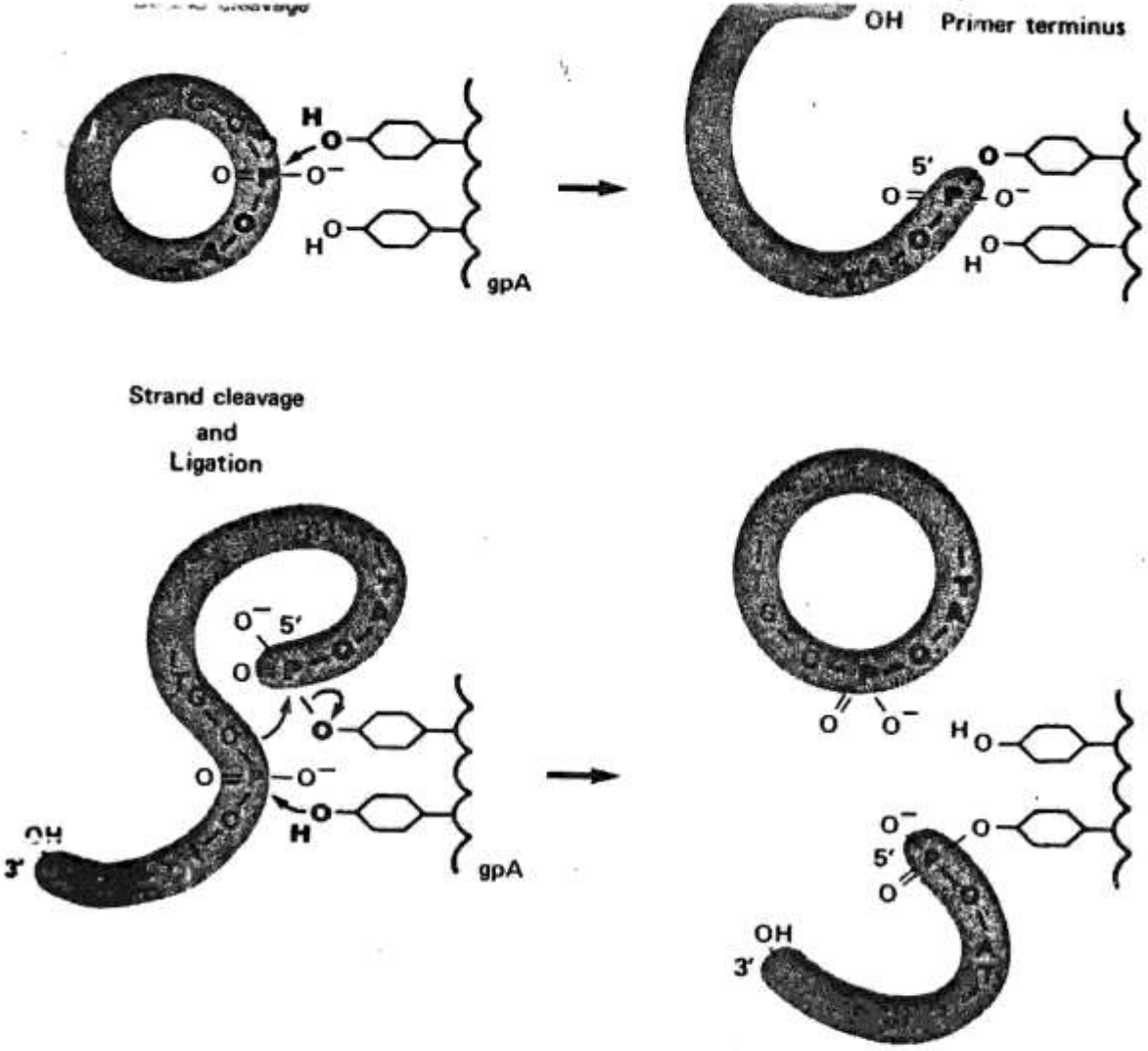
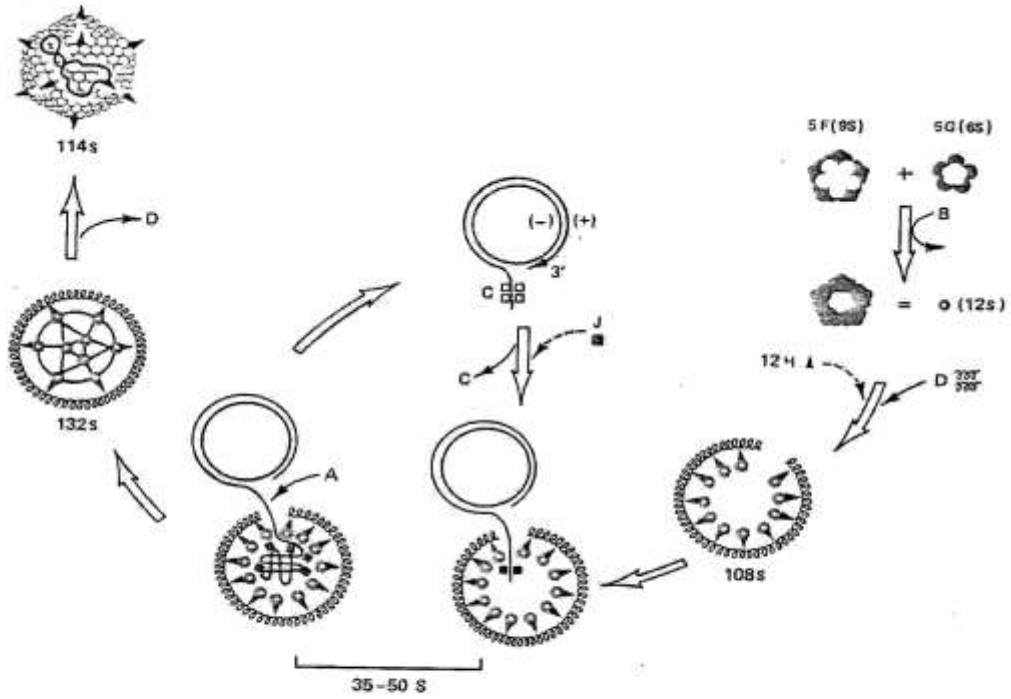
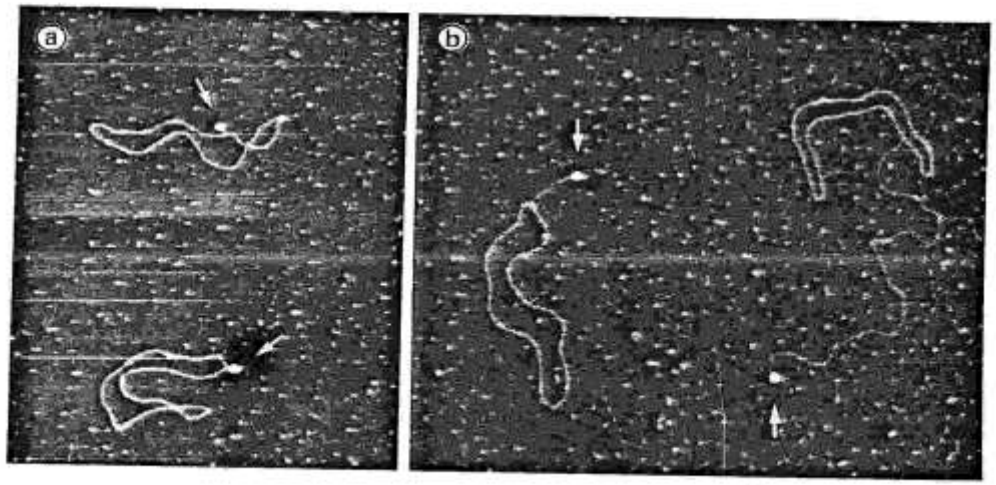


Figure 8-14
Model for the gpA-catalyzed cleavage and cleavage–ligation reactions which occur during initiation and termination of ϕX rolling-circle replication. The origin sequence, shown schematically as -TG-O-P-O-AT-, is in the viral (+) strand of the duplex rolling-circle intermediate shown in Fig. 8-13.

The ϕX174 DNA Packaging Reaction





The Replication Cycle of ϕX174

Table 17-8
Replication cycle of ϕX174

	Stage	Time, (minutes, at 33°)	Events
1	SS→RF	0–1	adsorption and penetration; viral SS→parental RF; transcription of RF
2	RF→RF	1–20	parental RF→~60 progeny RF
		25	RF multiplication stops; host DNA synthesis stops
3	RF→SS	20–30	~35 rolling circles→~500 viral SS→phage particles
		40	cell lysis

104. Kornberg A (1978) *CSHS* 43:1; Meyer RR, Shlomai J, Kobori J, Bates D, Rowen L, McMacken R, Ueda K, Kornberg A (1978) *CSHS* 43:289; Eisenberg S, Scott JF, Kornberg A (1978) *CSHS* 43:295.

The Replication Proteins of E. coli used by ϕX174

TABLE 11-5
Replication proteins of *E. coli* used by phage ϕX174

Polypeptide	Mass kdal	Subunits	Function	Unamplified yield: molecules/cell	Unamplified yield: mg/kg[a]	Amplification[d]
SSB	74	4	single-strand binding	300	20	
protein i	80	4	prepriming	150	0.5	
protein n	25	1	prepriming			
protein n′	75	1	site recognition, ATPase	80	0.3	
protein n″	11	1	prepriming			
dnaC	29	1	prepriming			
dnaB	250–300	4–6	mobile promoter, ATPase	20	0.3	10–100
primase	60	1	primer formation	100	0.2	
holoenzyme[c]-α	140	1				
β	40	1				
γ[b]	52	1				10
δ	32	1	synthesis	20	0.5	
ε	25	1				
θ	10	2				
pol I	109	1		300	10	70
ligase	74	1	ligation	300	10	500
gyrase	400	4				
nalA(A)	210	2	supertwisting			
cou(B)	190	2				
rep	65	1	helicase	50	0.6	10
dUTPase	64	4	dUTPase	350	3	

[a]Mg protein/kg wet weight of cells.
[b]*dnaZ* (γ polypeptide)
[c]See Table 5-3 for more details.
[d]Normal protein level was increased this many times by introducing a plasmid or phage vector containing the encoding gene.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