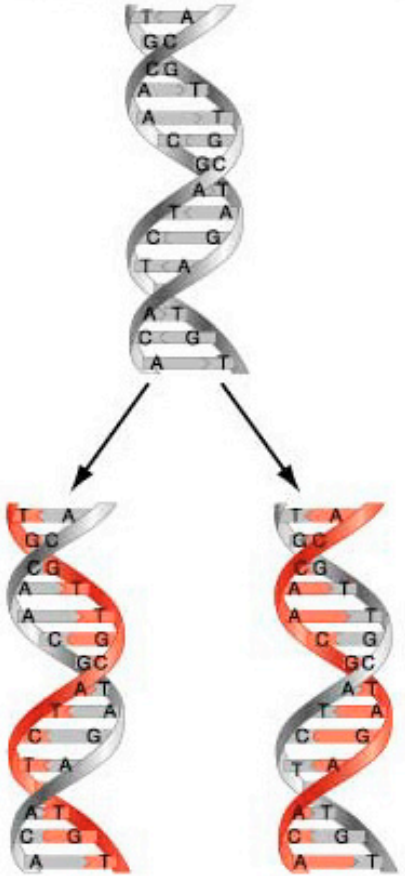
DNA REPLICATION

MODELS OF DNA REPLICATION

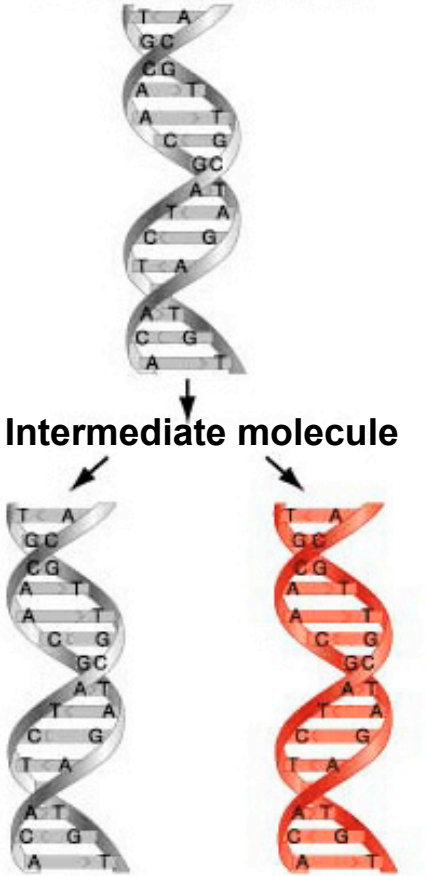
(a) Hypothesis 1:

Semi-conservative replication



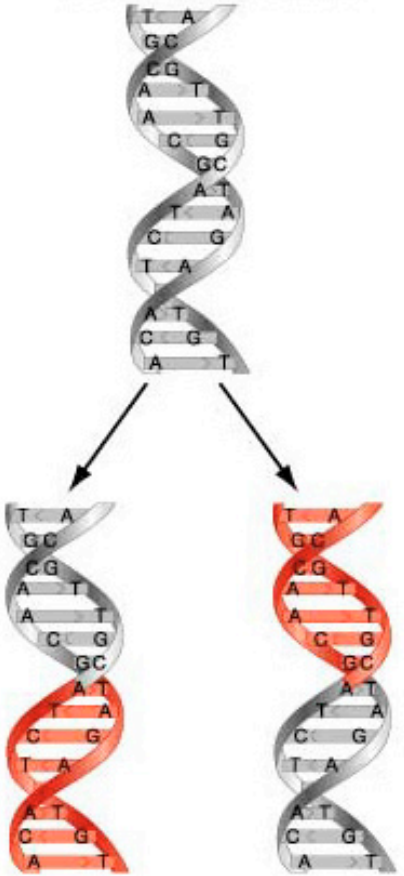
(b) Hypothesis 2:

Conservative replication



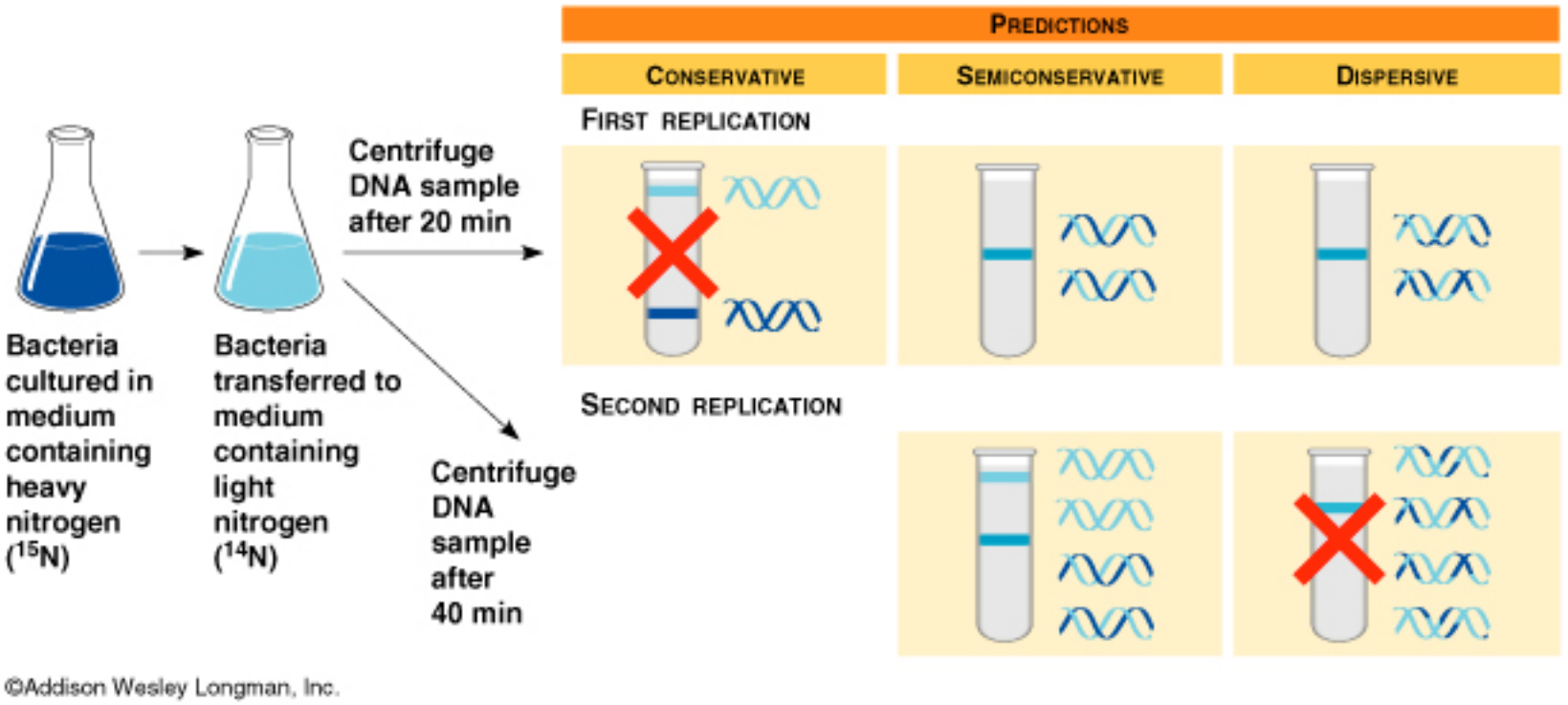
(c) Hypothesis 3:

Dispersive replication

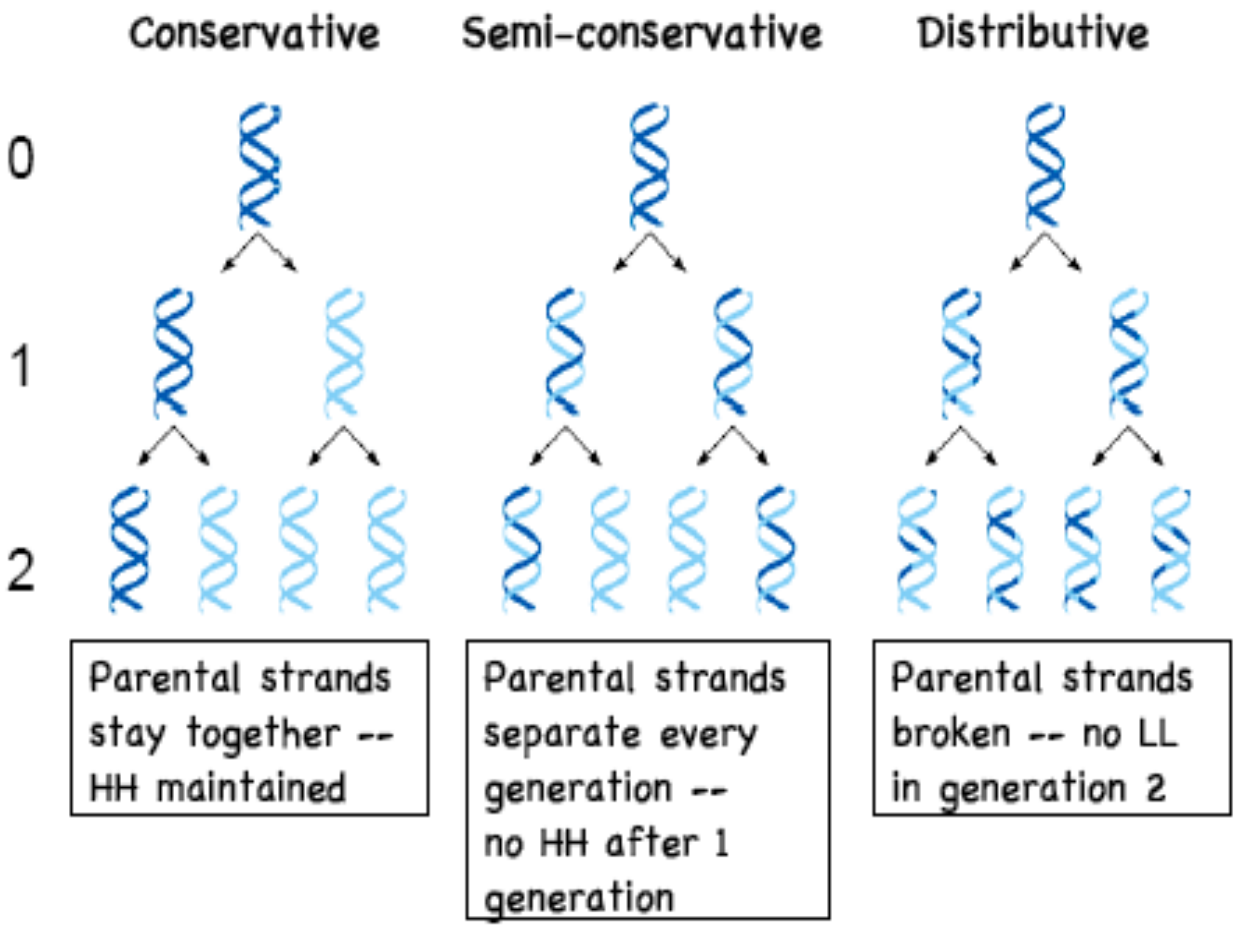


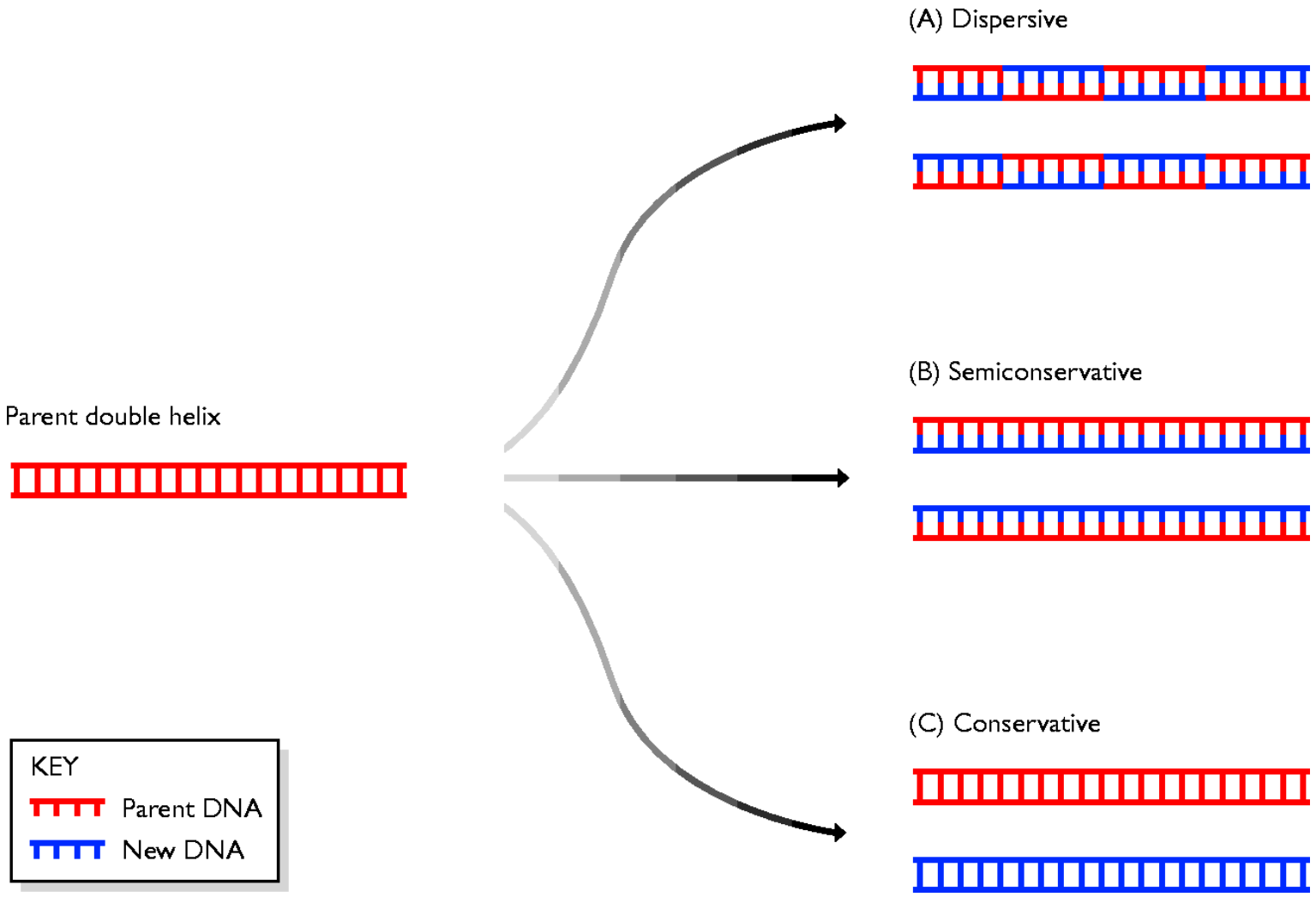
Meselson and Stahl

Semi-conservative replication of DNA

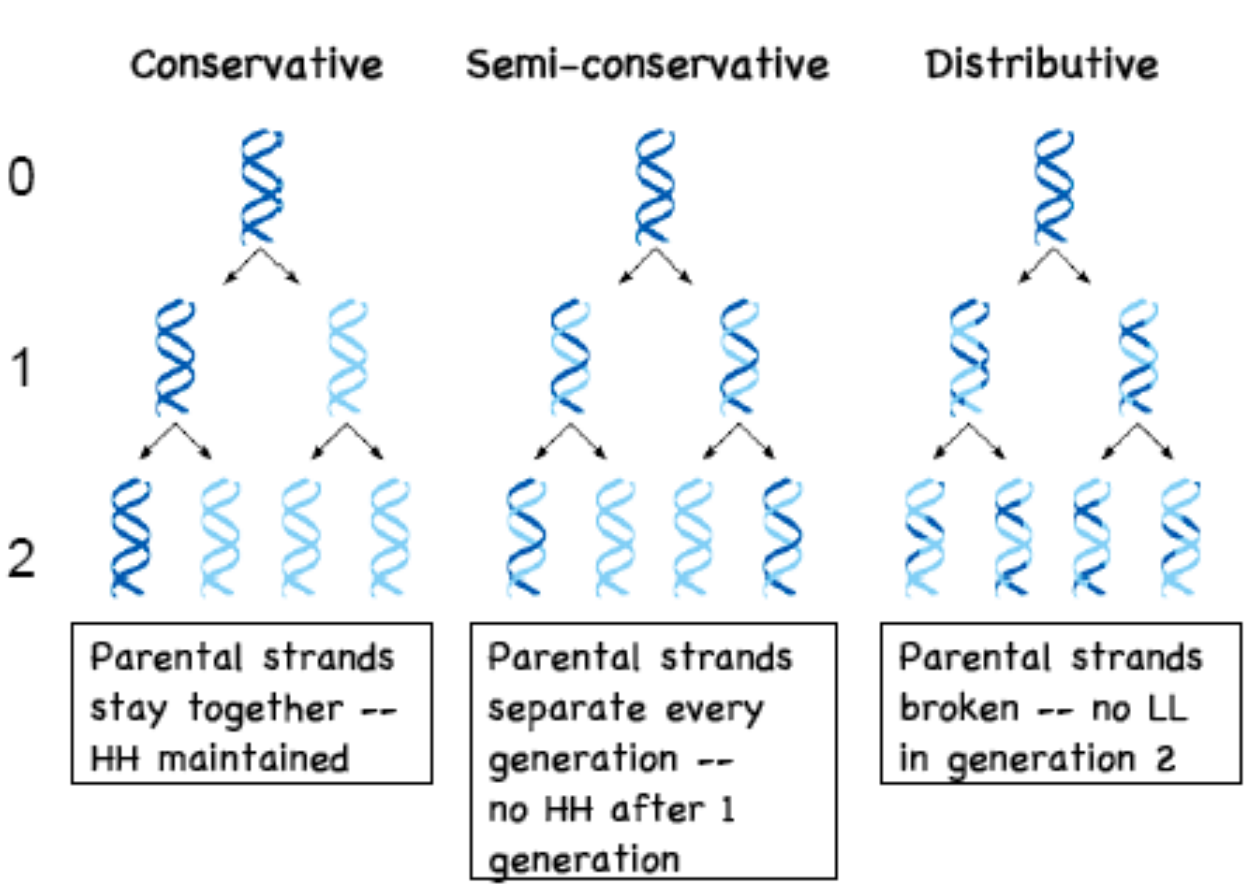


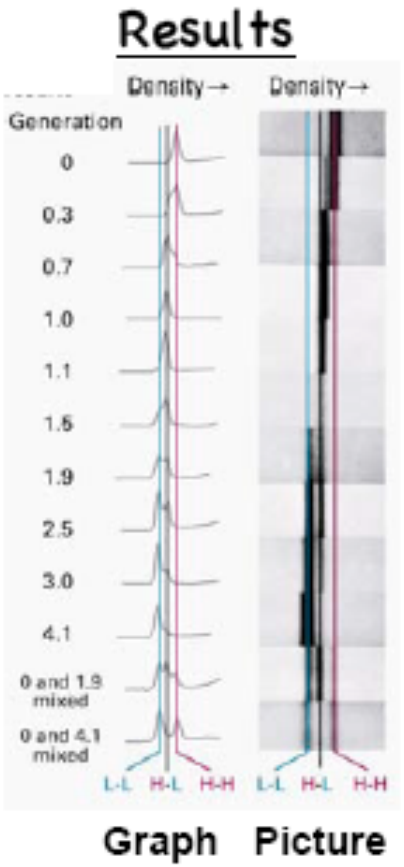
Predictions of Meselson-Stahl experiment



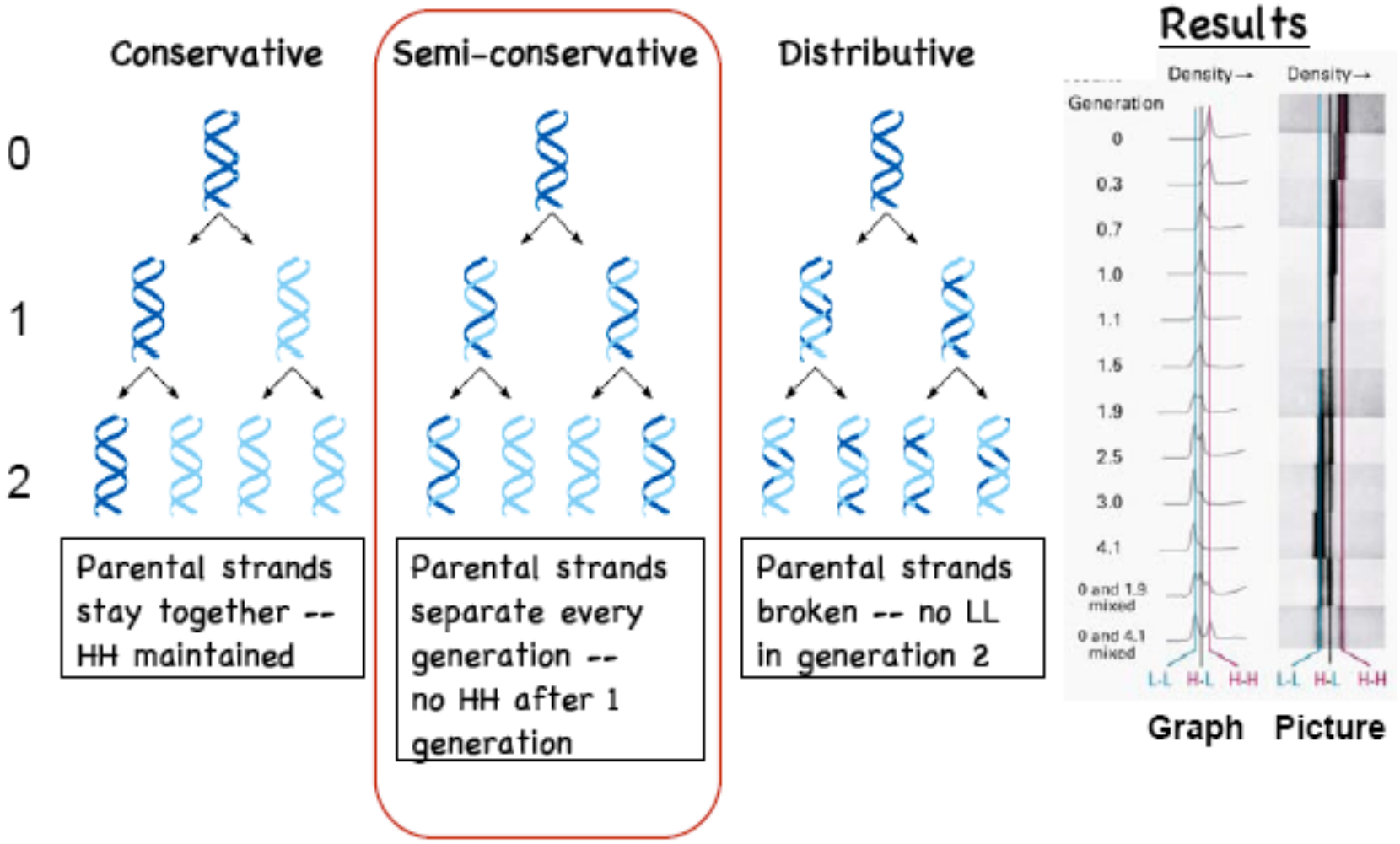

(A) Dispersive
Parent double helix
(B) Semiconservative
(C) Conservative
KEY
Parent DNA
New DNA

Results of Meselson-Stahl experiment

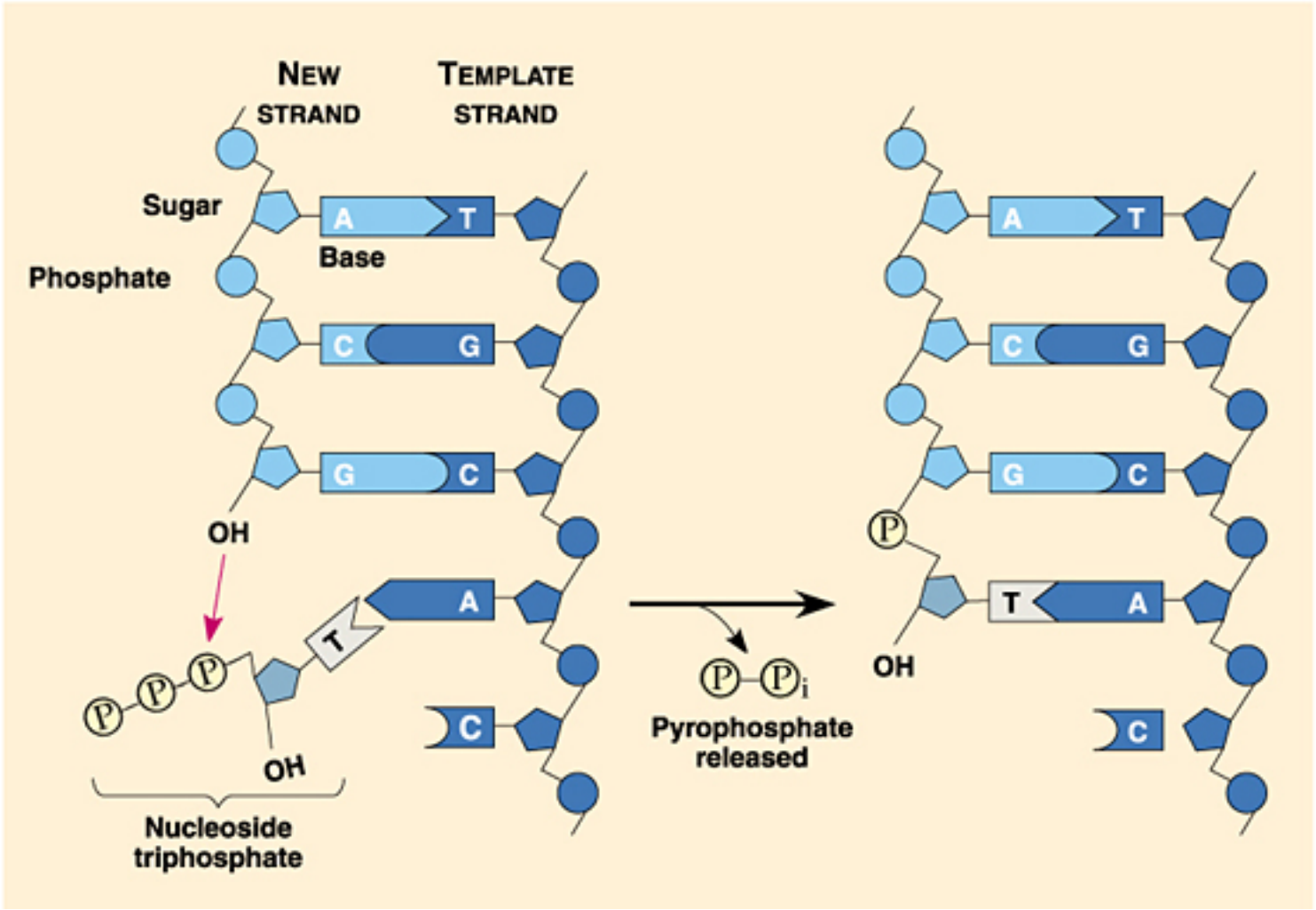




DNA replication is semi-conservative



Each “parent” strand must therefore act as a template for a new strand



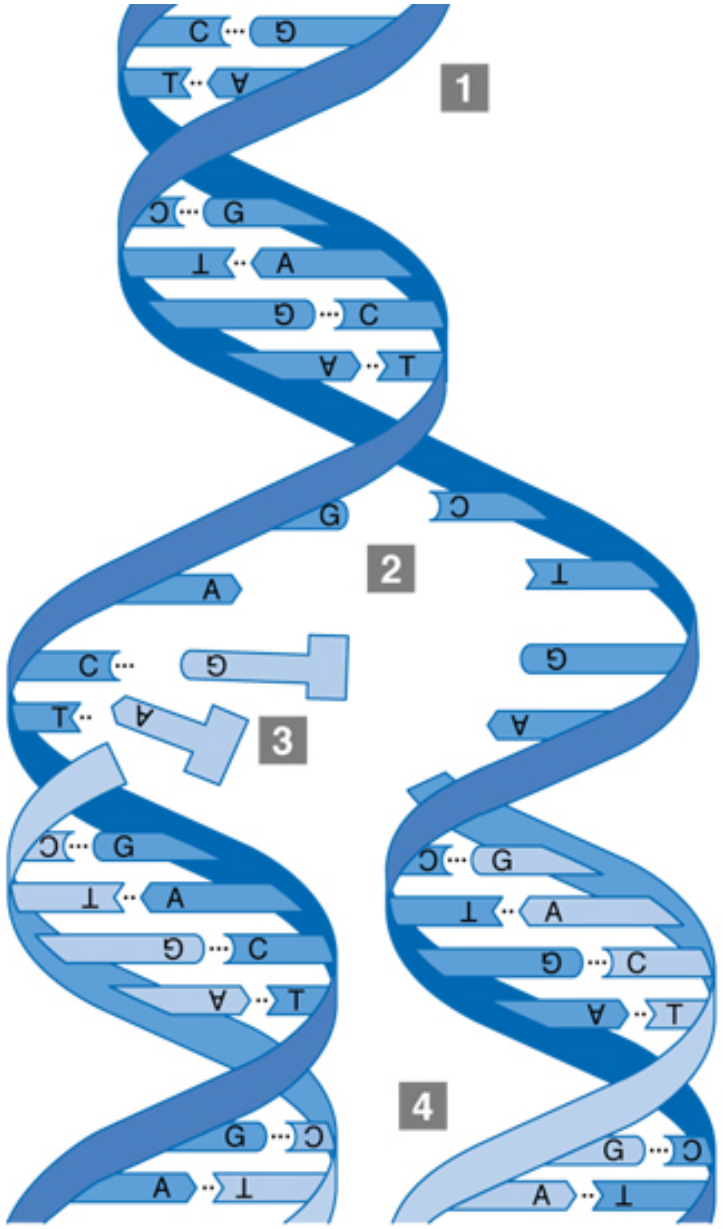
Nucleotides are successively added using deoxynucleoside triphosphosphates (dNTP’s)

Double-stranded DNA must be unwound before it is copied

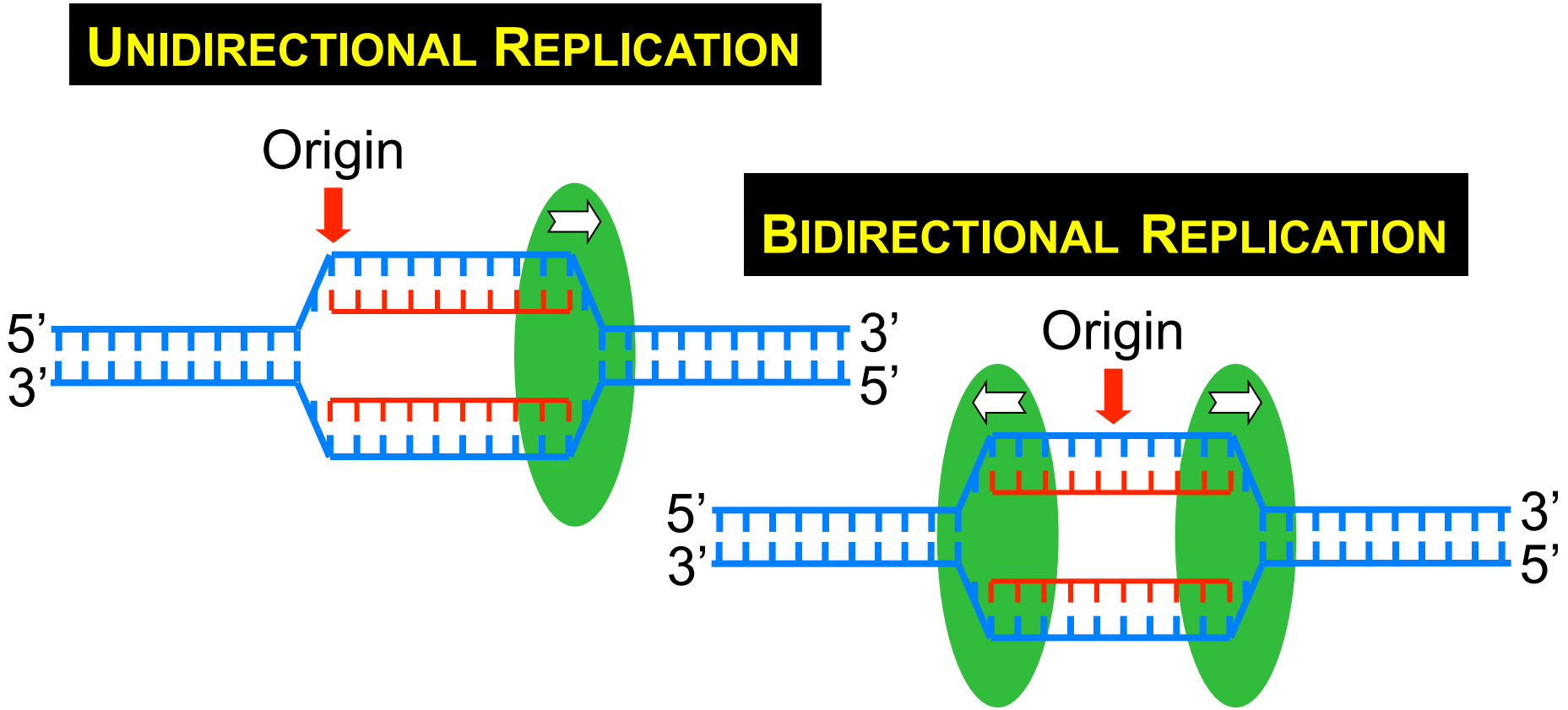
The junction of the unwound molecules is a replication fork.

A new strand is formed by pairing complementary bases with the old strand.

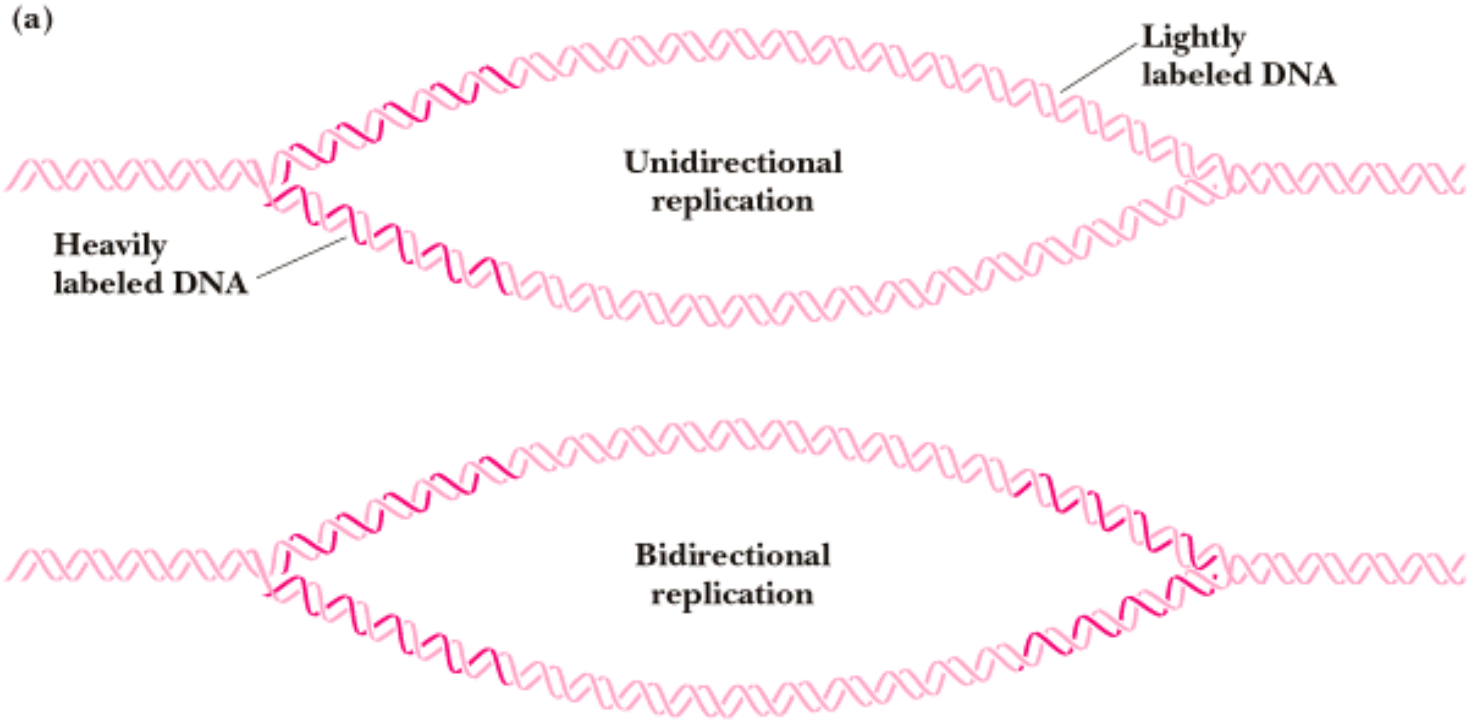
Two molecules are made. Each has one new and one old DNA strand.



Is Replication Uni- or Bidirectional?



Evidence points to bidirectional replication



Label at both replication forks

Proteins of DNA Replication

DNA exists in the nucleus as a **condensed, compact structure**. To prepare DNA for replication, a series of proteins aid in the unwinding and separation of the double-stranded DNA molecule.
These proteins are required because DNA must be single-stranded before replication can proceed.

1. **DNA Helicases** - These proteins bind to the double stranded DNA and stimulate the separation of the two strands.

2. **DNA single-stranded binding proteins** - These proteins bind to the DNA as a tetramer and stabilize the single-stranded structure that is generated by the action of the helicases. Replication is 100 times faster when these proteins are attached to the single- stranded DNA.

3. **DNA Topoisomerase** - This enzyme catalyzes the formation of negative supercoils that is thought to aid with the unwinding process.

In addition to these proteins, **several other enzymes** are involved in bacterial DNA replication.

4. DNA Polymerase - DNA Polymerase I (Pol I) was the first enzyme discovered with polymerase activity, and it is the best characterized enzyme. Although this was the first enzyme to be discovered that had the required polymerase activities, it is not the primary enzyme involved with bacterial DNA replication. That enzyme is DNA Polymerase III (Pol III).
Three activities are associated with *DNA polymerase I;*

* 5' to 3' elongation (polymerase activity)
* 3' to 5' exonuclease (proof-reading activity)
* 5' to 3' exonuclease (repair activity)

The second two activities of DNA Pol I are important for replication, but DNA Polymerase III (Pol III) is the enzyme that performs the 5'-3' polymerase function.

5. Primase - The requirement for a free 3' hydroxyl group is fulfilled by the RNA primers that are synthesized at the initiation sites by these enzymes.

6. DNA Ligase - Nicks occur in the developing molecule because the RNA primer is removed and synthesis proceeds in a discontinuous manner on the lagging strand. The final replication product does not have any nicks because DNA ligase forms a covalent phosphodiester linkage between 3'-hydroxyl and 5'-phosphate groups.

Arthur Kornberg discovered DNA dependent DNA polymerase

Used an "*in vitro*" system: the classic biochemical approach

1. Grow *E. coli*
2. Lyse cells
3. Prepare extract
4. Fractionate extract
5. Search for DNA polymerase activity using an ASSAY

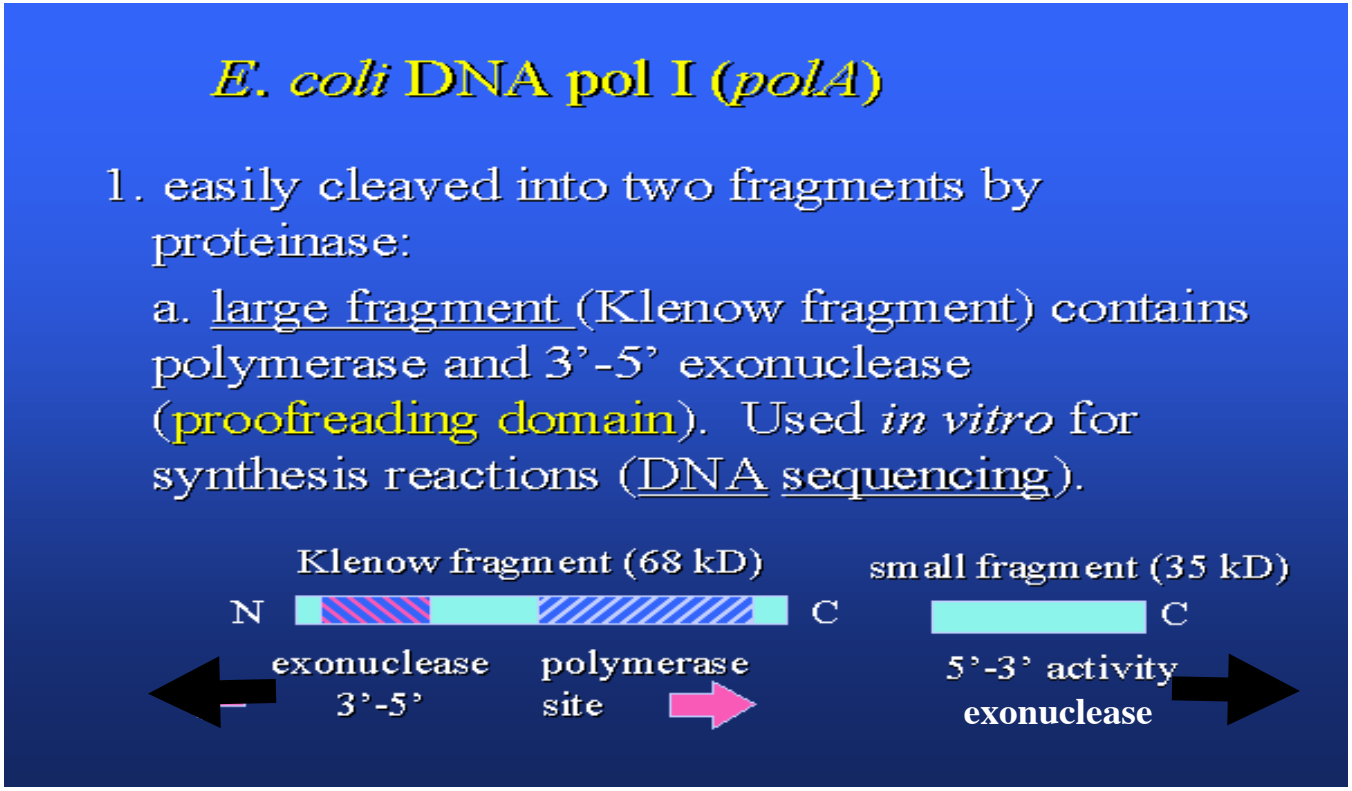
Requirements for DNA polymerase activity	
Template	[Basis for heredity]
dNTPs (not ATP, not NDPs, not NMPs)	[Building blocks]
Mg^{2+}	[Promotes reaction]
Primer - (complementary bases at 3' end, removed by fractionation and added back)	[DNA pol can't start!]

The discovery of DNA polymerase.

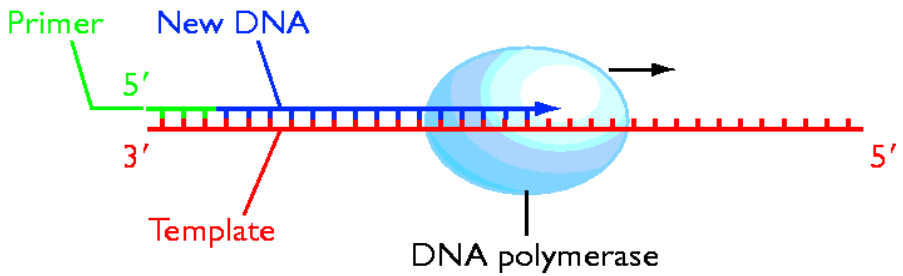
Arthur Kornberg and Bob Lehman pursued an enzyme in bacterial extracts that would elongate a chain of deoxyribonucleic acid just like glycogen synthase elongates a chain of glycogen.

The enzymatic activity was unusual:

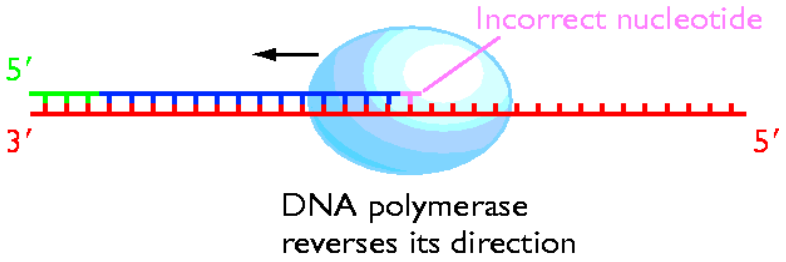
1) Needed a template which dictates what nucleotide was added: substrate was directing enzymatic activity
2) Needed a primer annealed to the template.



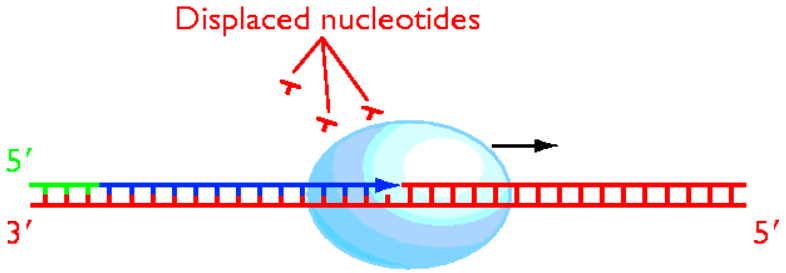
(A) 5′→3′ DNA synthesis



(B) 3′→5′ exonuclease activity



(C) 5′→3′ exonuclease activity



The DNA Polymerase Family

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

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

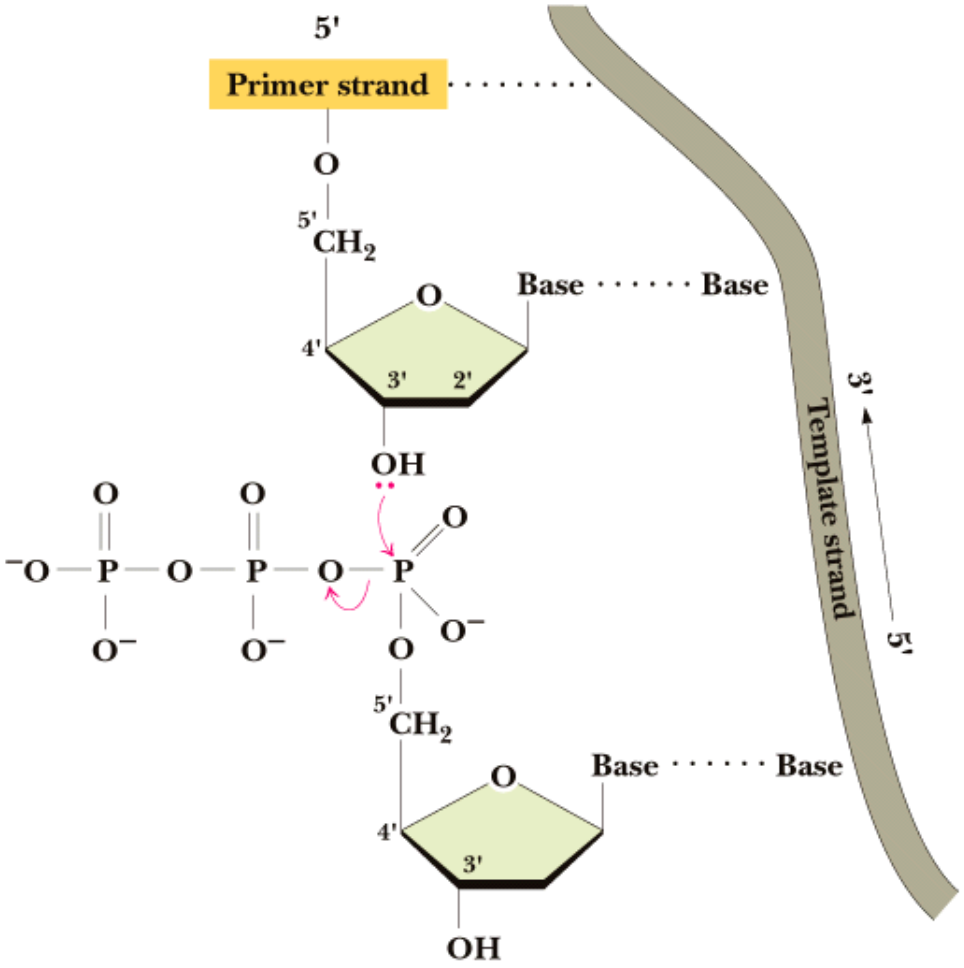
DNA Pol I

DNA Polymerase I has *THREE* different enzymatic activities in a single polypeptide:

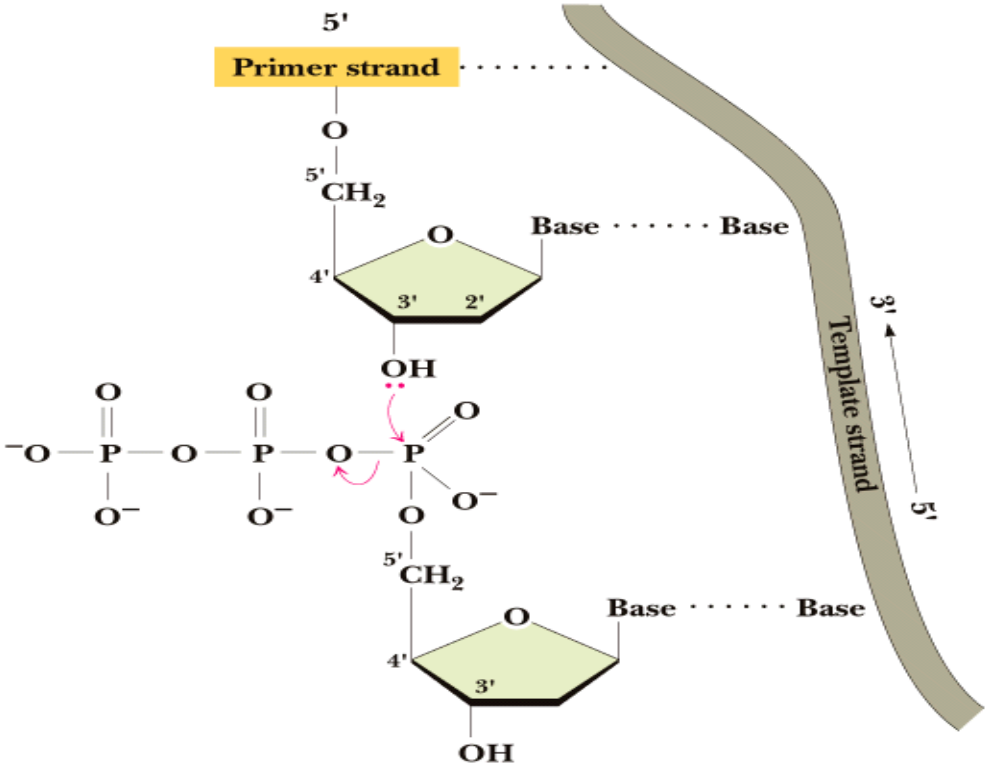
- a 5’ to 3’ DNA polymerizing activity
- a 3’ to 5’ exonuclease activity
- a 5’ to 3’ exonuclease activity

DNA polymerases add nucleotides to the 3'OH end of the growing strand. Strand elongation is therefore always 5' to 3'

Subsequent hydrolysis of PPi drives the reaction forward



DNA polymerase mechanism



Each dNTP provides the nucleophile (3'-OH) for the next round

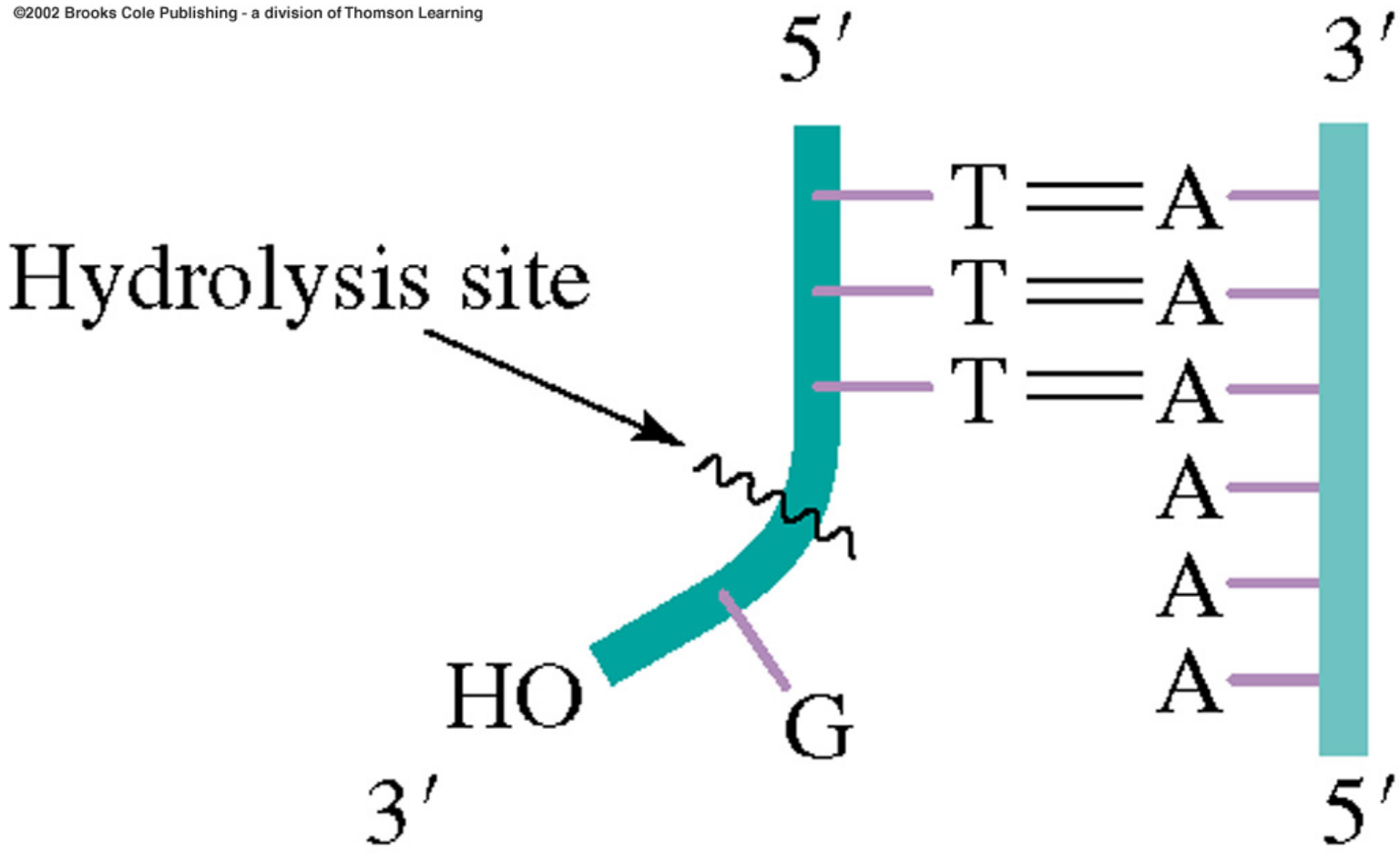
Why the exonuclease activities?

- The 5’–3’ exonuclease activity removes bases from in front of the enzyme, so that DNA pol I can replace existing polymer.

- The 3'-5' exonuclease activity removes incorrectly matched bases, so that the polymerase can try again.

Figure 11.7a Exonuclease activity of DNA polymerase I

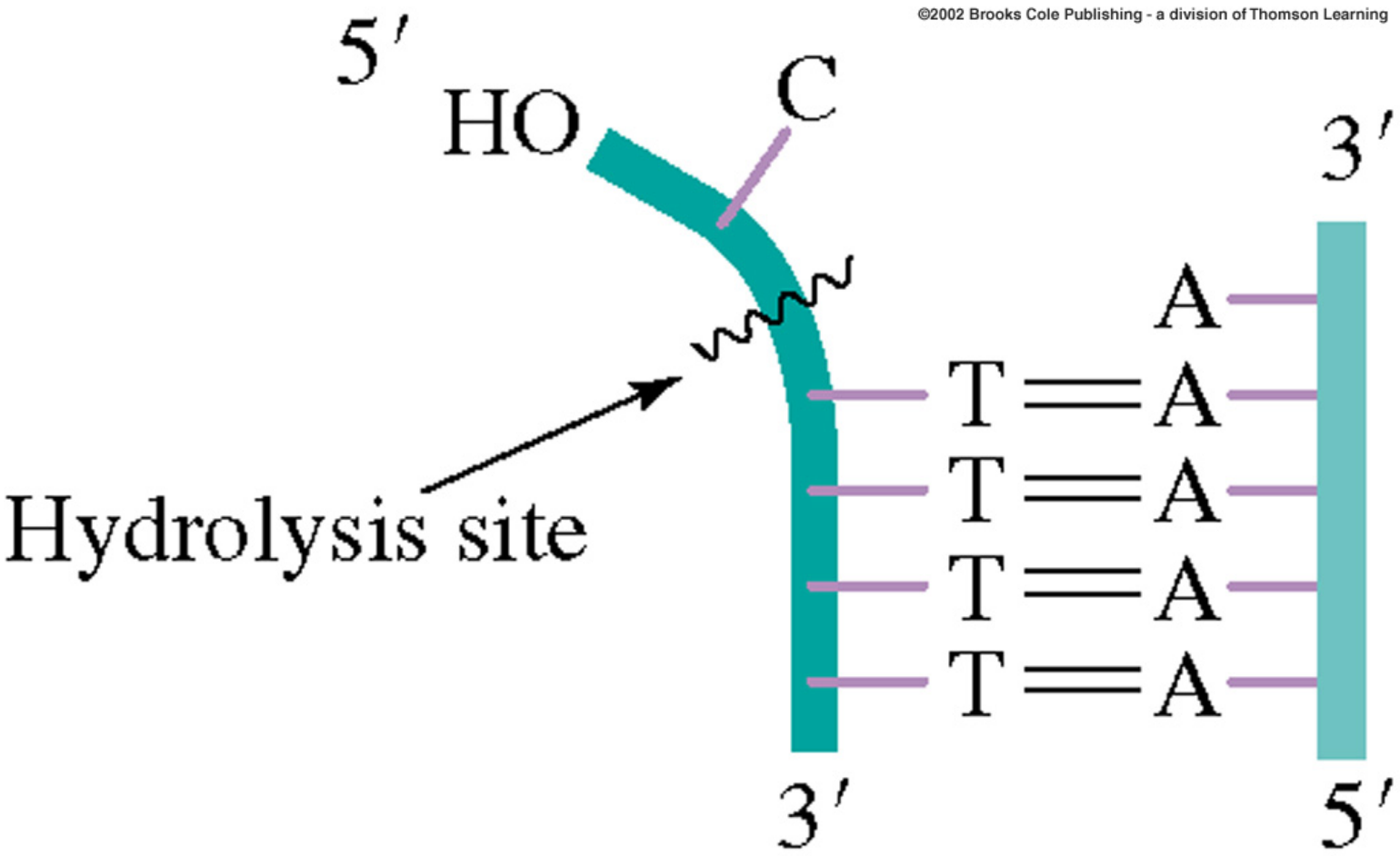
- **(a) 3’→5’ exonuclease activity**



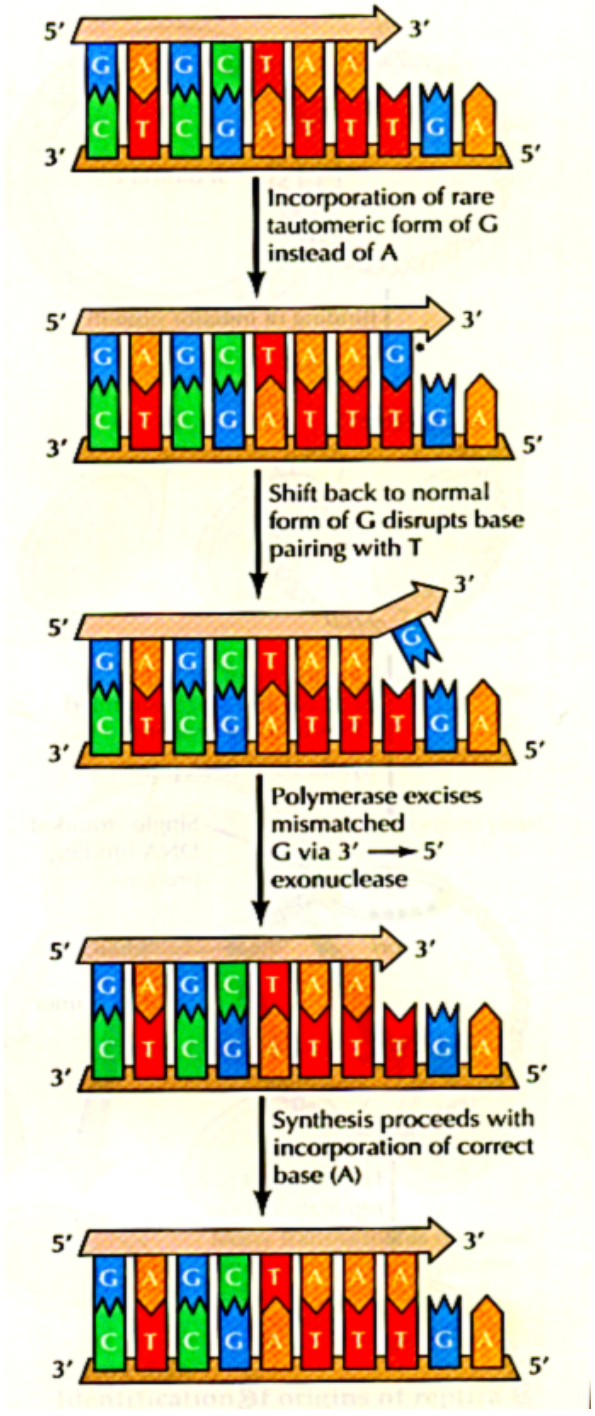
DNA polymerase I acts as a proofreading and repair enzyme by catalyzing hydrolytic removal of mismatched bases.

Figure 11.7b Exonuclease activity of DNA polymerase I

- **(b) 5’→3’ exonuclease activity**



DNA polymerase I acts as a proofreading and repair enzyme by catalyzing hydrolytic removal of mismatched bases.



Proofreading activity of the 3’ to 5’ exonuclease.

DNA Pol I stalls if the incorrect nucleotide is added - it can’t add the next one in the chain

Proof reading activity is slow compared to polymerizing activity, but the **stalling** of DNA Pol I after insertion of an incorrect base allows the proofreading activity to catch up with the polymerizing activity and remove the incorrect base.

DNA Polymerase III

The "real" replicative polymerase in E. coli

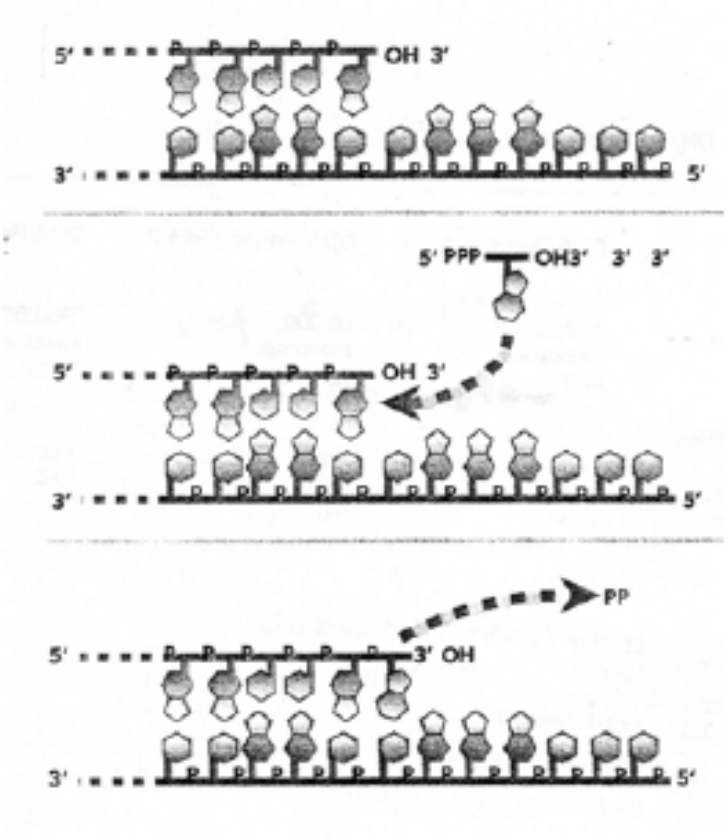
- It's fast: up to 1,000 dNTPs added/sec/enzyme
- It's highly processive: >500,000 dNTPs added before dissociating
- It's accurate: makes 1 error in 10^7 dNTPs added,
- Has 3'-5' exonuclease "proofreading" (like Pol I), this gives a final error rate of 1 in 10^{10} overall.

Nobel Prize for DNA polymerase I

Comparison of DNA Polymerases of *E. coli*

	DNA polymerase		
	I	II	III
Structural gene*	*pol*A	*pol*B	*pol*C (*dna*E)
Subunits (number of different types)	1	≥4	≥10
M_r	103,000	88,000†	830,000
3′→5′ Exonuclease (proofreading)	Yes	Yes	Yes
5′→3′ Exonuclease	Yes	No	No
Polymerization rate (nucleotides/sec)	16–20	40	250–1,000
Processivity (nucleotides added before polymerase dissociates)	3–200	1,500	≥500,000

DNA polymerase activities -- 5'-->3' nucleotide addition



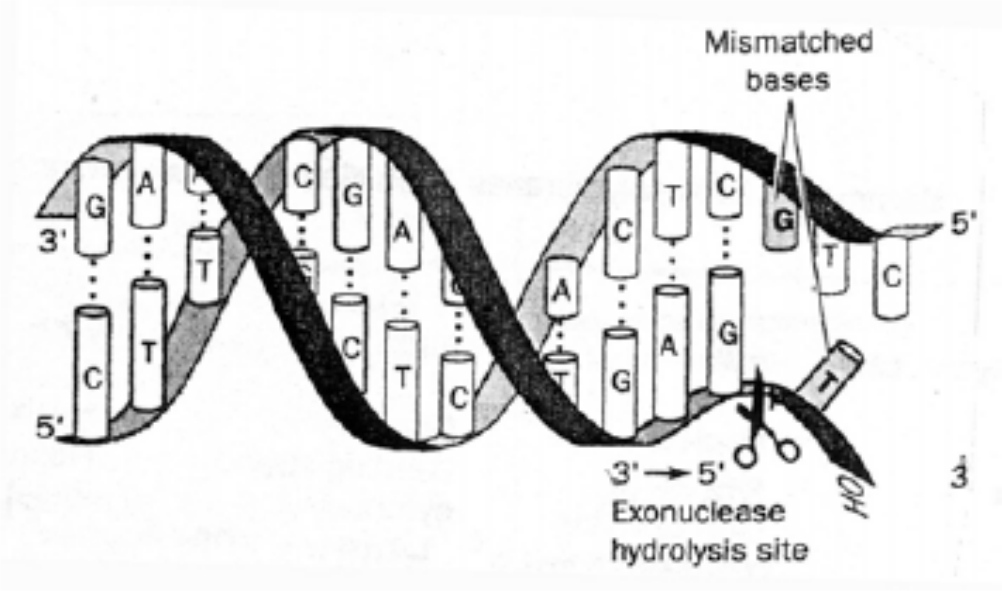
Primer has a free 3'-OH

Incoming dNTP has a 5' triphosphate

Pyrophosphate (PP) is lost when dNMP adds to the chain

DNA polymerase reactions -- editing

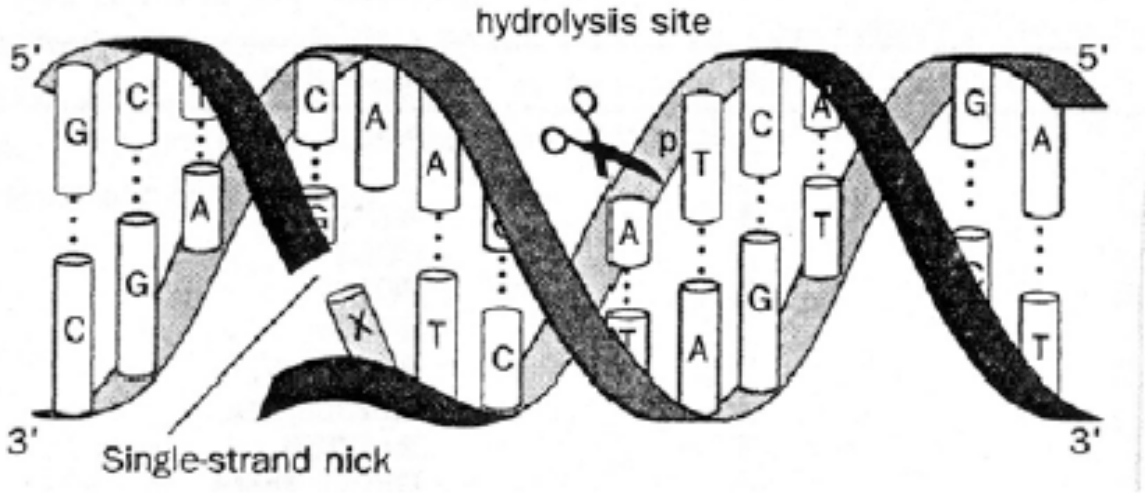
3'-->5' exonuclease



Opposite reaction compared to polymerase
(But no PPi used or dNTP made)

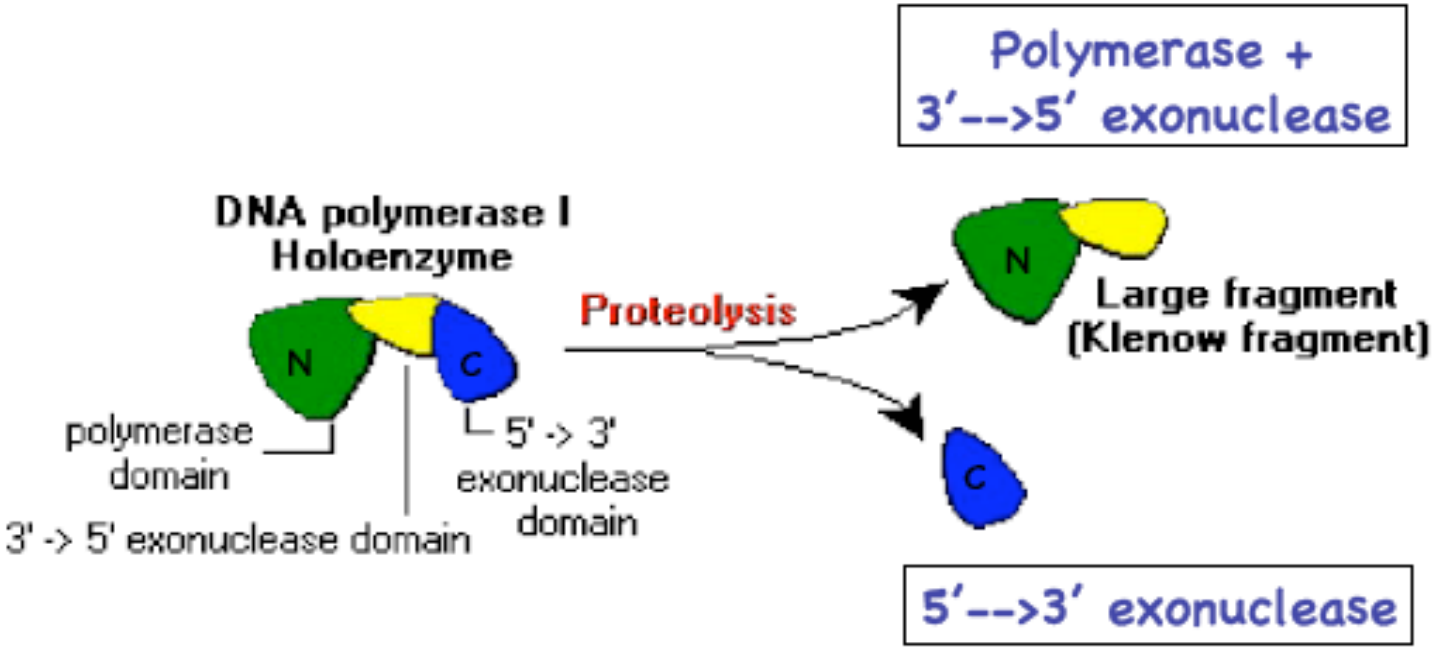
DNA polymerase reactions -- nick translation

5'-->3' exonuclease

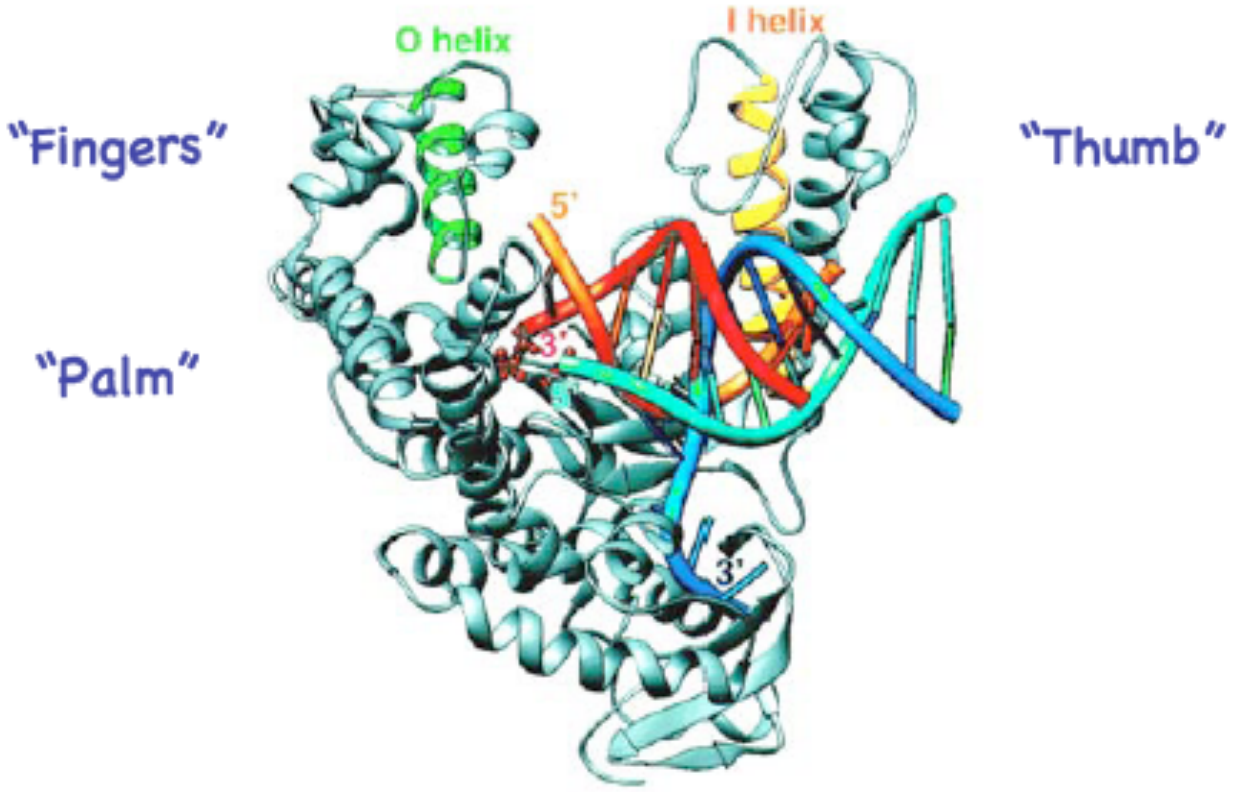


Creates single-stranded template in front for repair

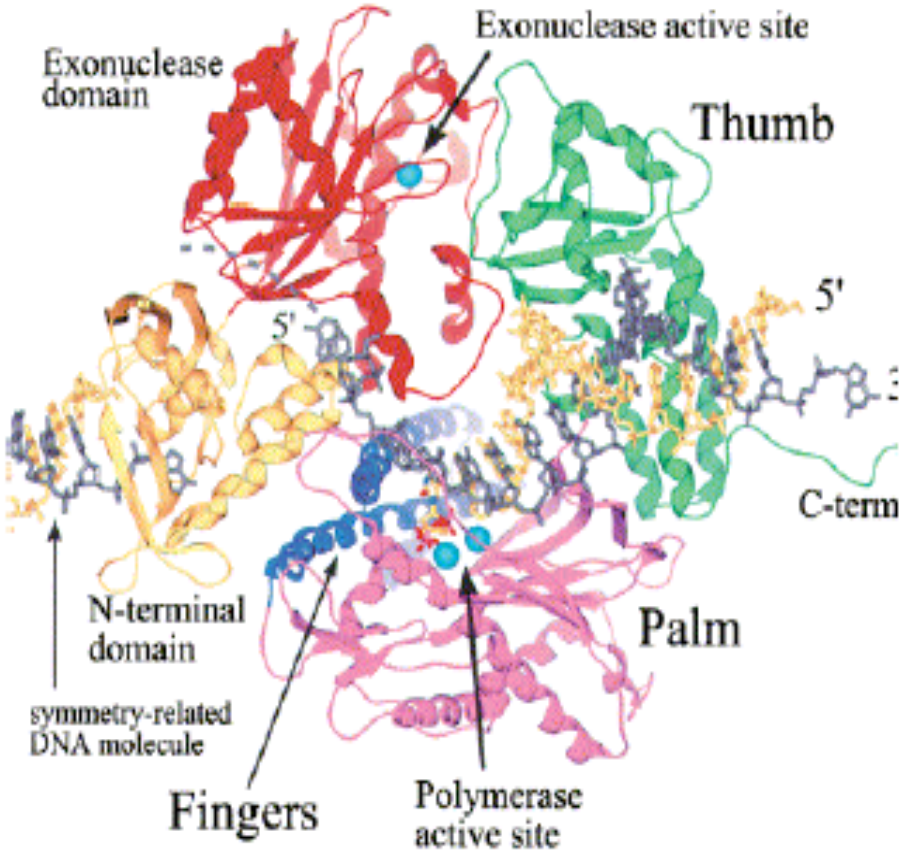
DNA pol I Klenow fragment lacks 5'-->3' exonuclease



Structure of the DNA complex of the Klenow fragment of DNA pol I

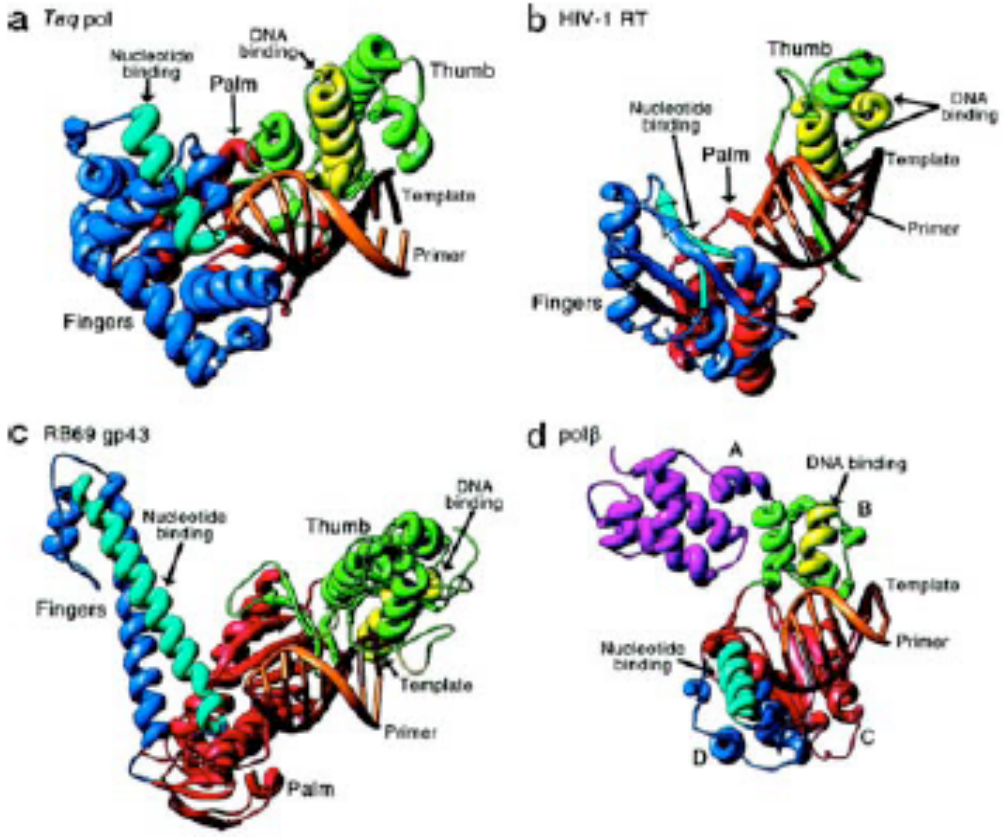


Functional sites in RB69 DNA polymerase + primer-template + dTTP

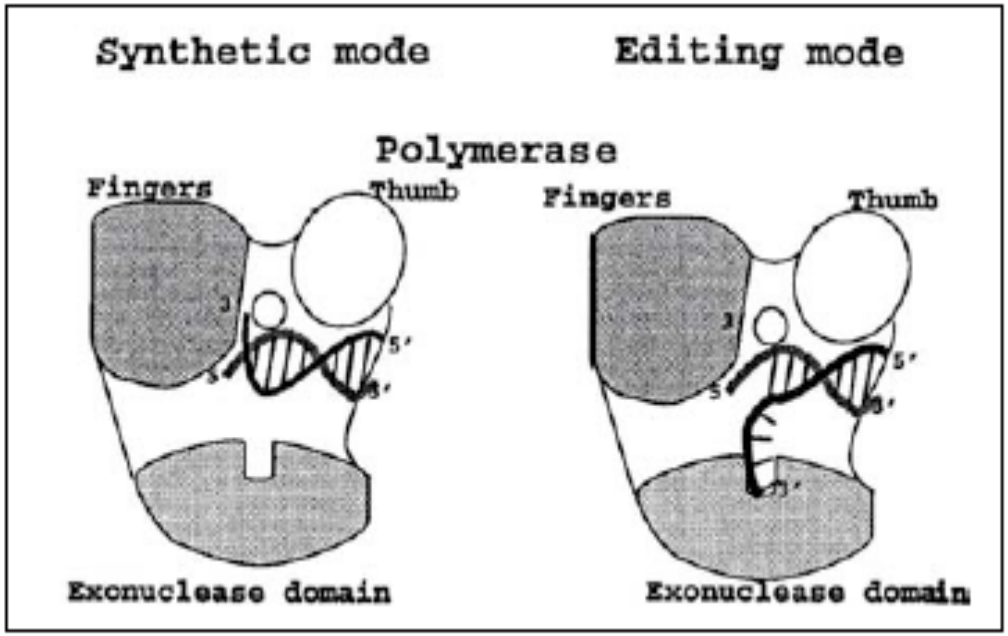


Fold conserved in DNA polymerases

"Fingers" "Palm" "Thumb"



Two different active sites for nucleotide addition and 3'--> 5" exonuclease



Polymerization is a race against the 3'-->5' exonuclease

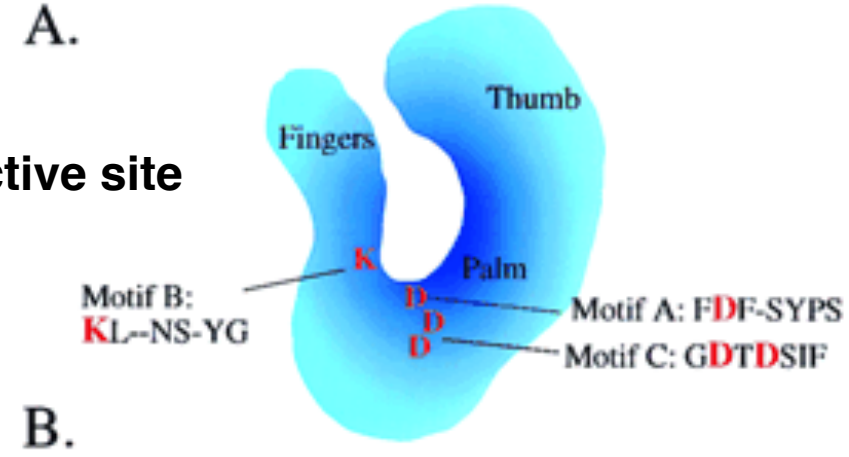
Relative rates of addition and exonuclease control net reaction

Fidelity is due to the race between the polymerase and exonuclease

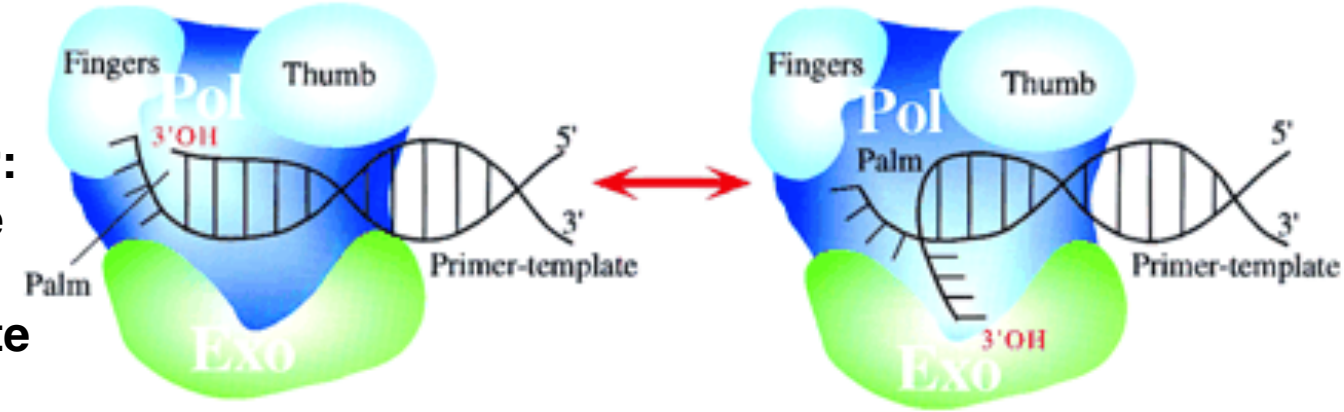
Model: Rate of nucleotide addition dominates the net reaction.

Structure of a DNA polymerase (gp43 from phage RB69)

Side view:
Polymerase active site



Top view with template-primer:
Polymerase site
And
proofreading site



There are many other proteins involved in DNA replication in *E. coli*

Protein Name	Function
DNA Gyrase	Unwinding DNA
SSB	Single-stranded DNA binding
DnaA	Initiation factor
HU	Histone-like (DNA binding and bending)
PriA	Primosome assembly
PriB	Primosome assembly
PriC	Primosome assembly
DnaB	DNA unwinding (helicase)
DnaC	DnaB chaperone
DnaT	Assists DnaC in delivery of DnaB
Primase	Synthesis of an RNA primer
DNAP III holoenzyme	Elongation (DNA synthesis)
DNAP I	Excises RNA primer, fills in with DNA
Ligase	Covalently links Okazaki fragments
Tus	Termination

A General Model for DNA Replication

1. The DNA molecule is unwound and prepared for synthesis by the action of DNA gyrase, DNA helicase and the single-stranded DNA binding proteins.

2. A free 3'OH group is required for replication, but when the two chains separate no group of that nature exists. RNA primers are synthesized, and the free 3'OH of the primer is used to begin replication.

3. The replication fork moves in one direction, but DNA replication only goes in the 5' to 3' direction. This paradox is resolved by the use of Okazaki fragments. These are short, discontinuous replication products that are produced off the **lagging strand**. This is in comparison to the continuous strand that is made off the **leading strand**.

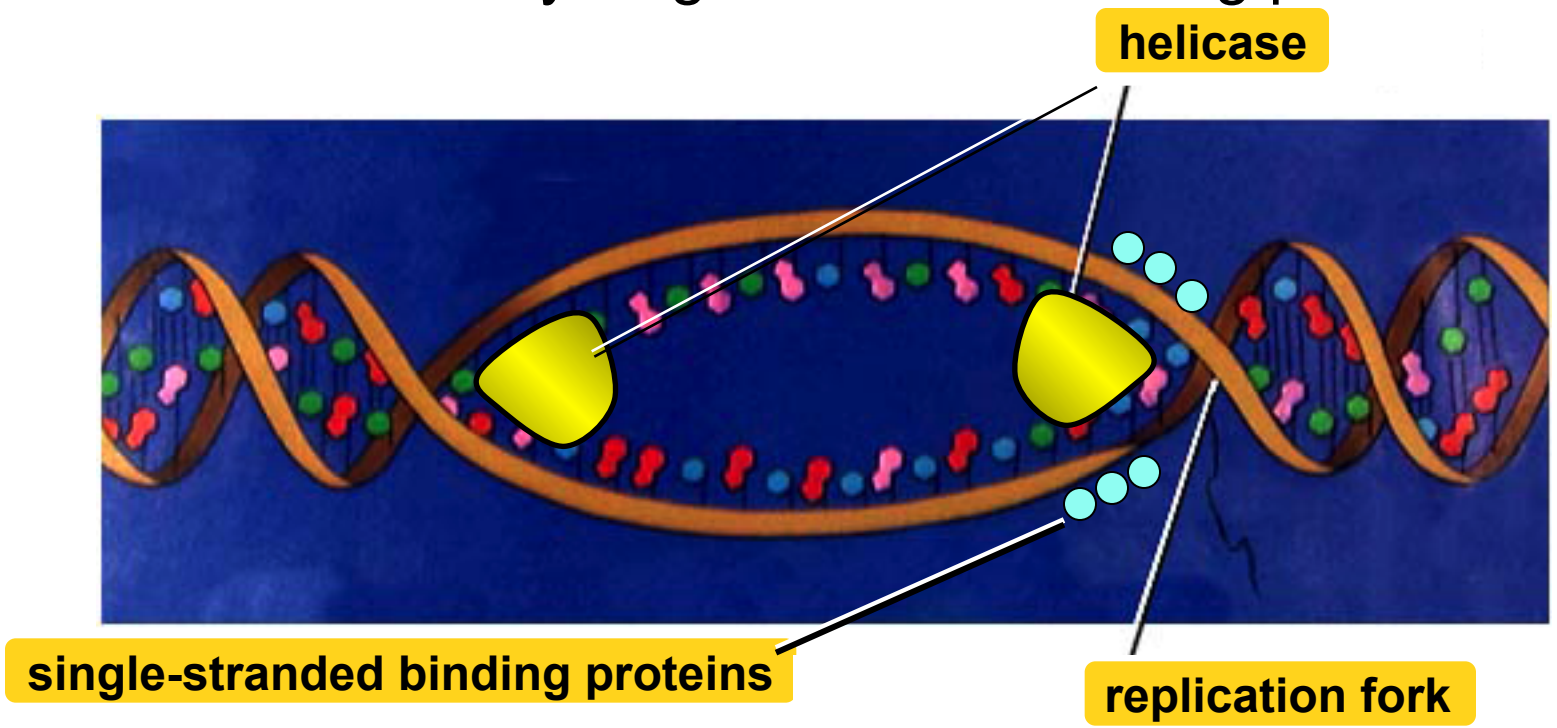
4. The final product does not have RNA stretches in it. These are removed by the **5' to 3' exonuclease** action of Polymerase I.

5. The final product does not have any gaps in the DNA that result from the removal of the RNA primer. These are filled in by the **5' to 3' polymerase** action of DNA Polymerase I.

6. DNA polymerase does not have the ability to form the final bond. This is done by the enzyme DNA **ligase**.

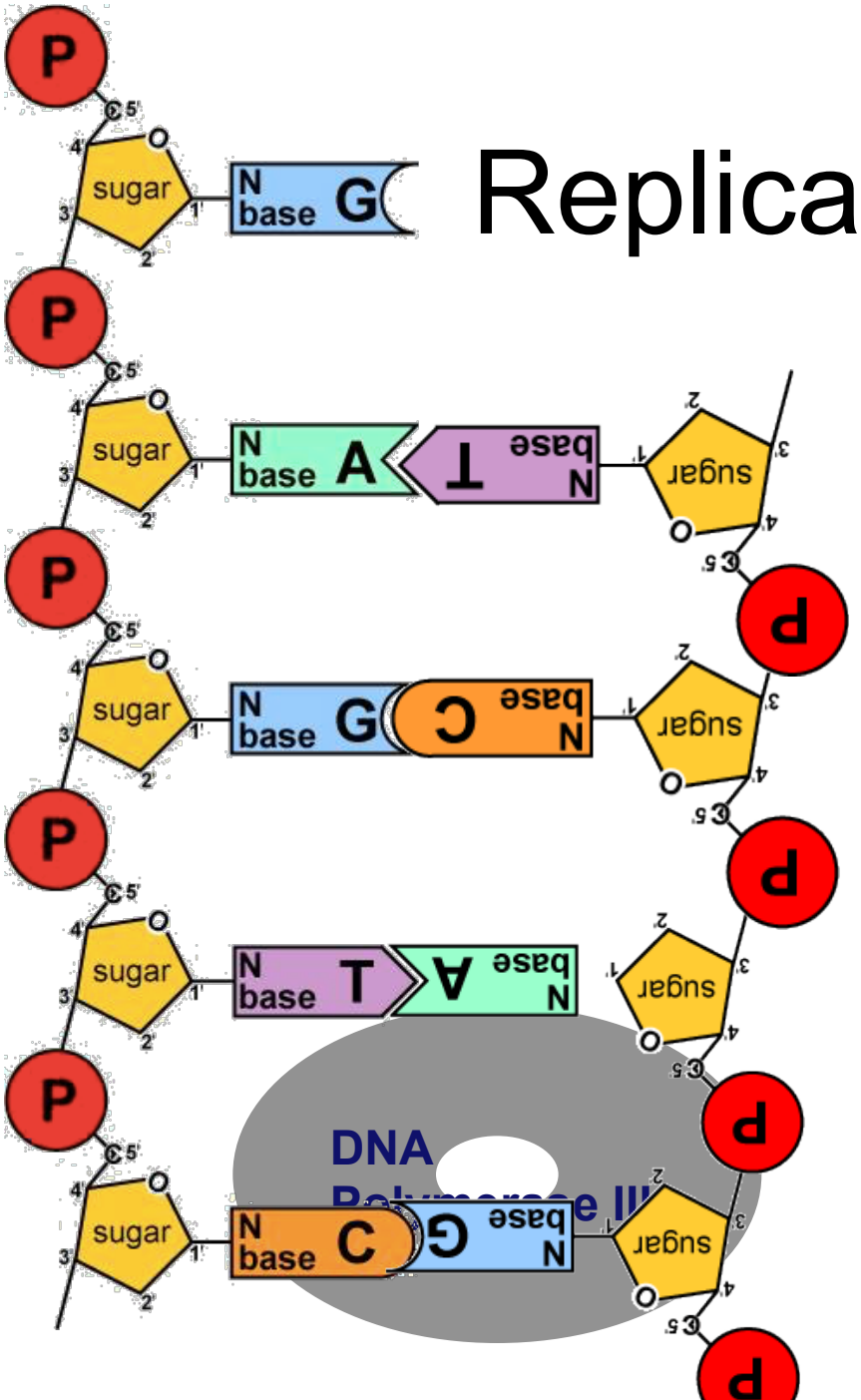
Replication: 1st step

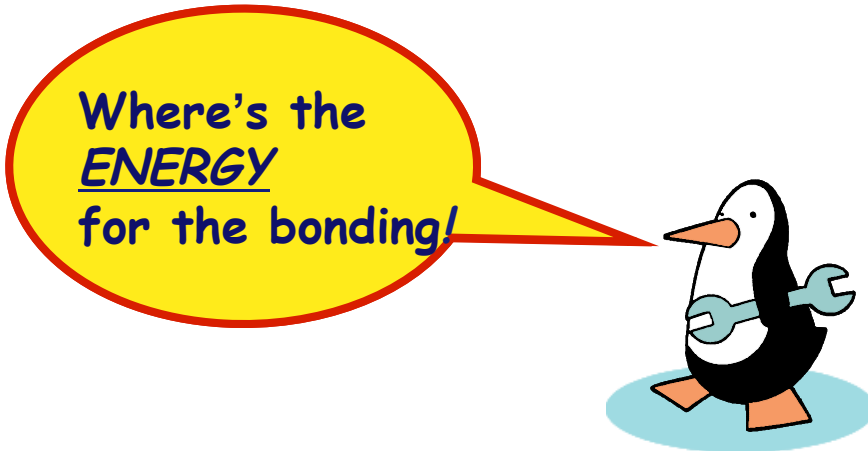
- Unwind DNA
  - helicase enzyme
    - unwinds part of DNA helix
    - stabilized by single-stranded binding proteins



Replication: 2nd step

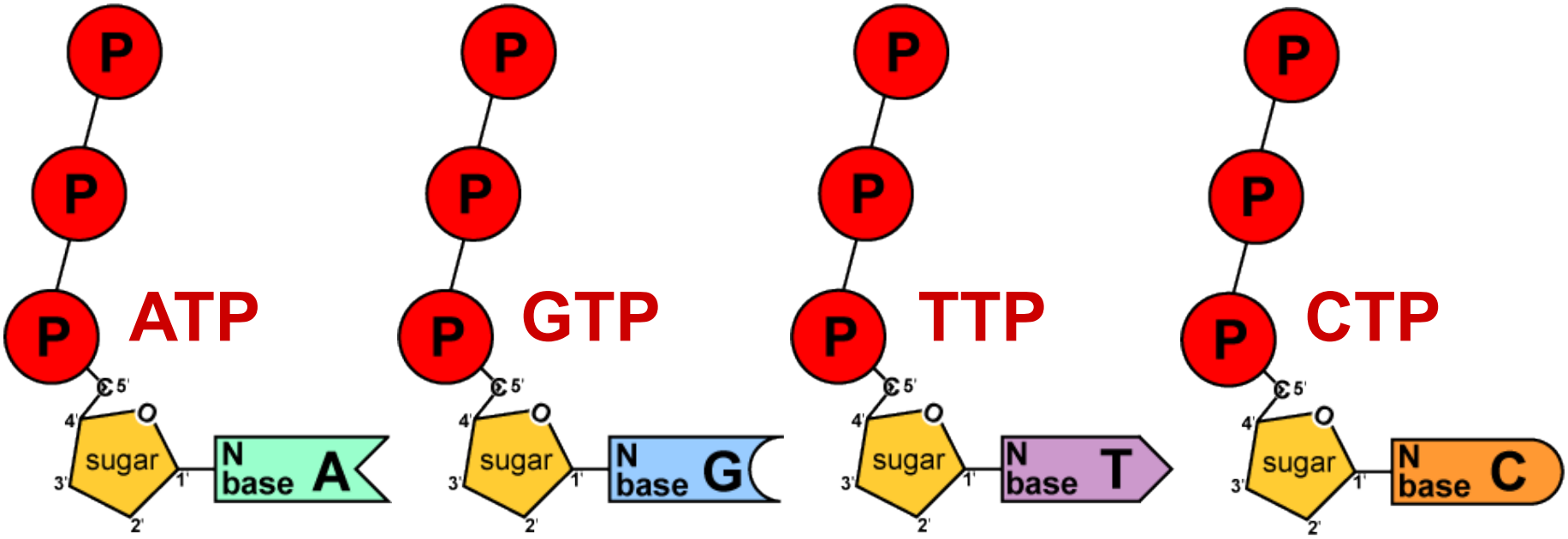
- **Build daughter DNA strand**
  - **add new complementary bases**
  - **DNA polymerase III**





Energy of Replication

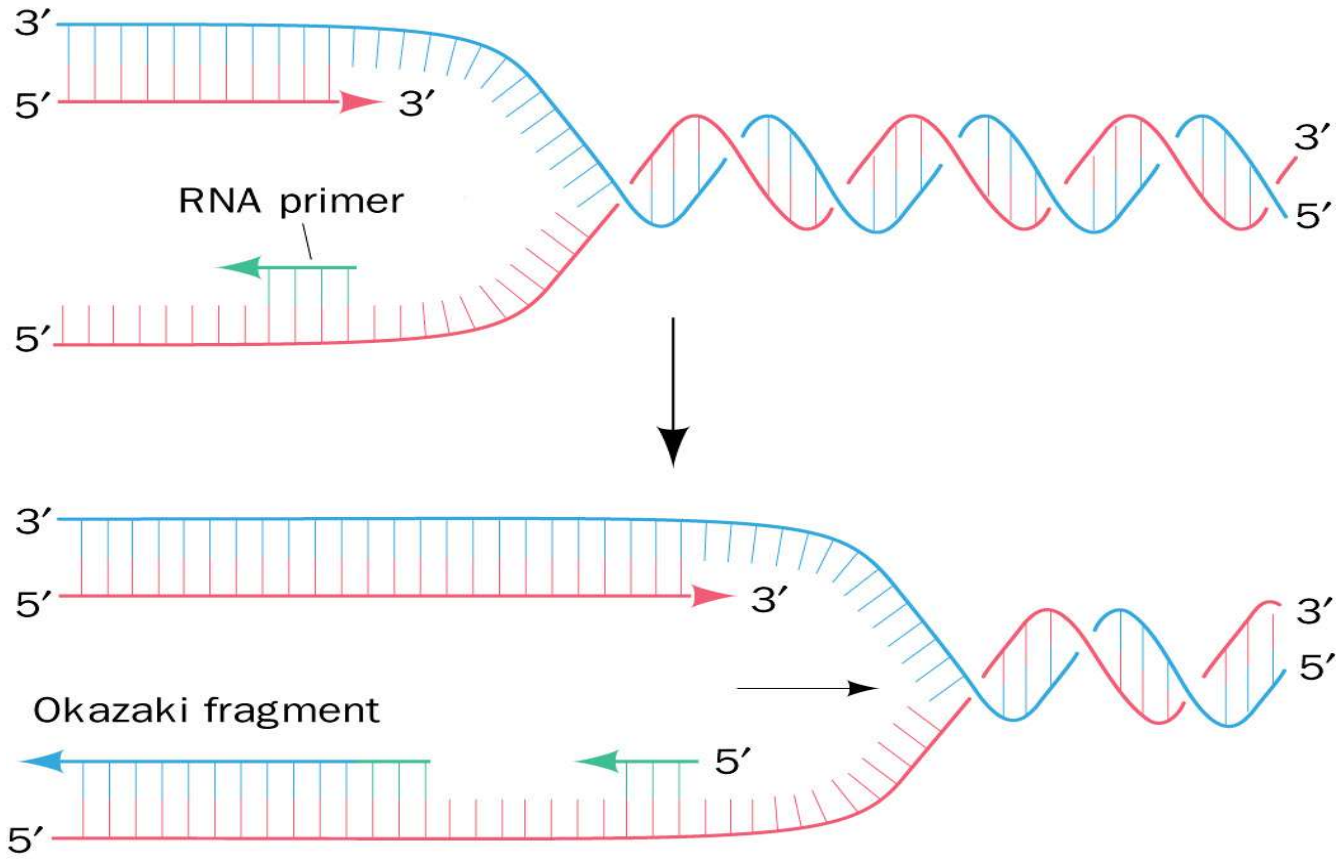
- The nucleotides arrive as nucleosides
  - DNA bases with P–P–P
    - P-P-P = energy for bonding
  - DNA bases arrive with their own energy source for bonding
  - bonded by enzyme: DNA polymerase III



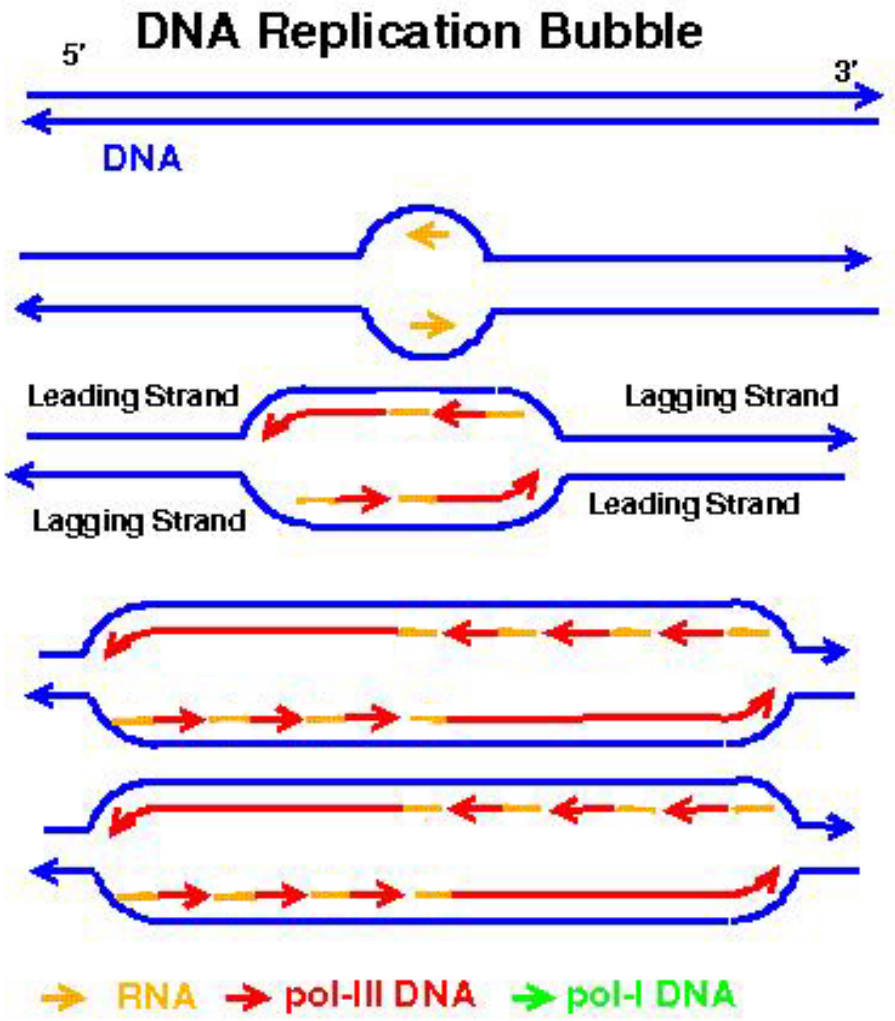
RNA Primers

- Need a 3’-OH group to extend DNA chain.
- Analysis of Okazaki fragments revealed that they have short (1-60 nt) RNA segments complementary to the template DNA.
- RNA primers catalyzed by 2 enzymes:
- RNA polymerase, large (459 kD), mediates transcription, rifampicin sensitive.
- Primase (DnaG), small (60 kD), rifampicin resistant.

Figure 30-7 Priming of DNA synthesis by short RNA segments.



RNA primed DNA replication



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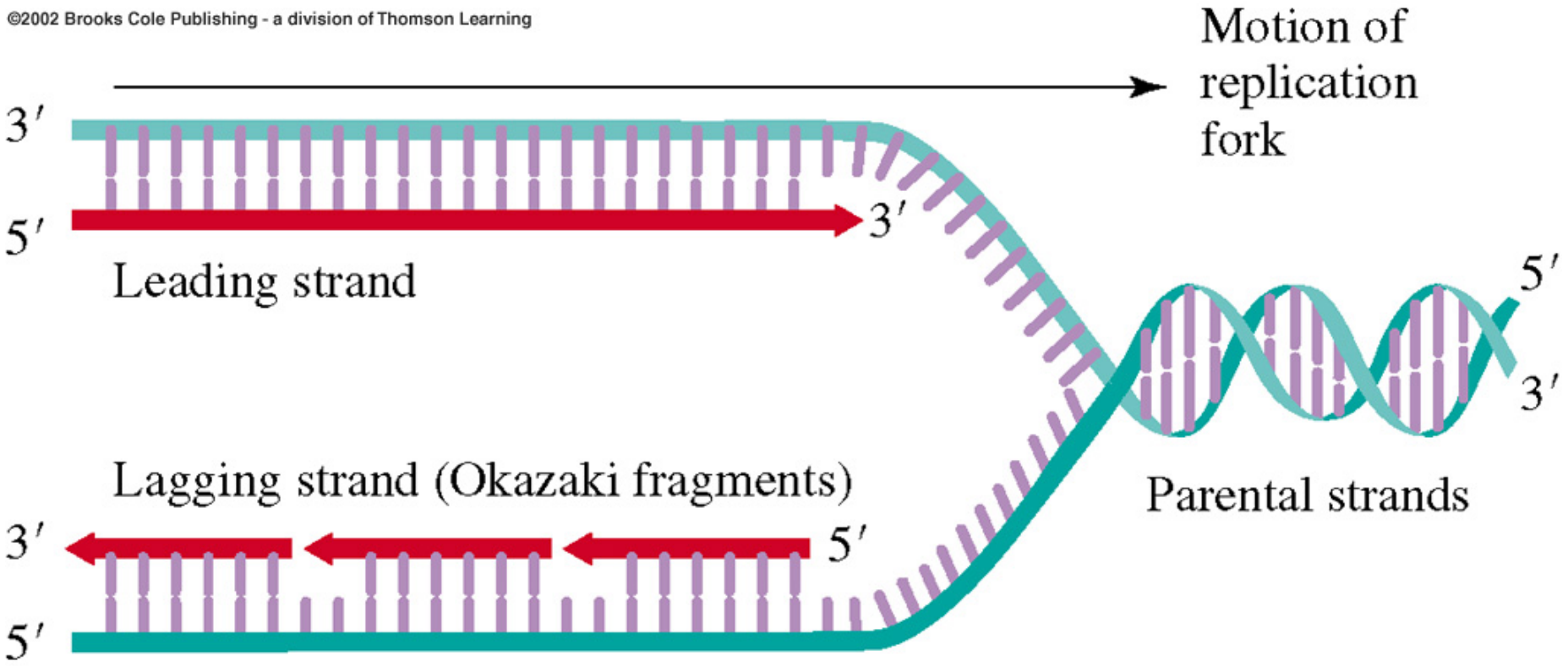
6. DNA polymerase does not have the ability to form the final bond. This is done by the enzyme DNA **ligase**.

Figure 11.8 Closeup of a replication fork showing initiation of the continuous leading strand and the discontinuous, lagging strand (Okazaki fragments)

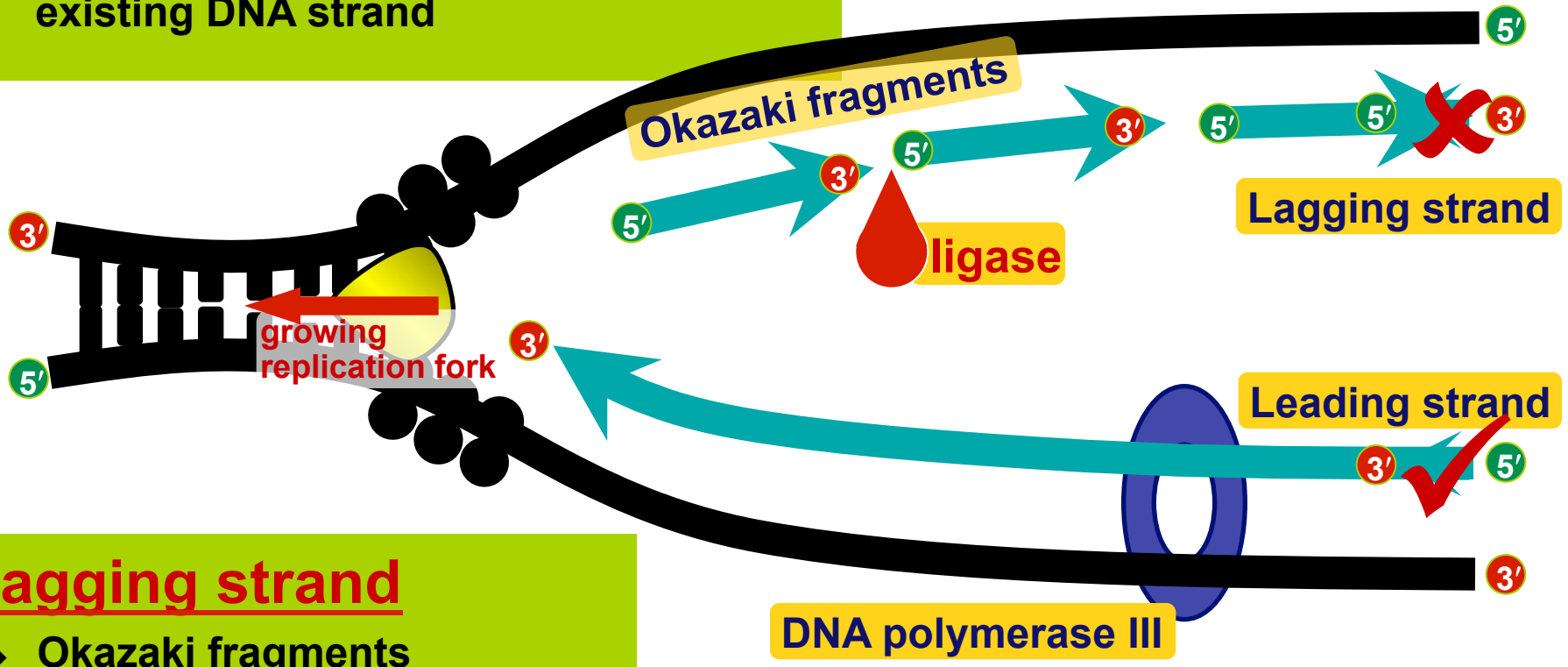


- All known DNA polymerases catalyze chain formation in the 5’ → 3’ direction.
- DNA strands must be copied in both directions!

Leading & Lagging str

Limits of DNA polymerase III

- can only build onto 3′ end of an existing DNA strand



Lagging strand

- Okazaki fragments
- joined by ligase
  - "spot welder" enzyme

Leading strand

- continuous synthesis

Looping the lagging strand to make both polymerases move in the same direction

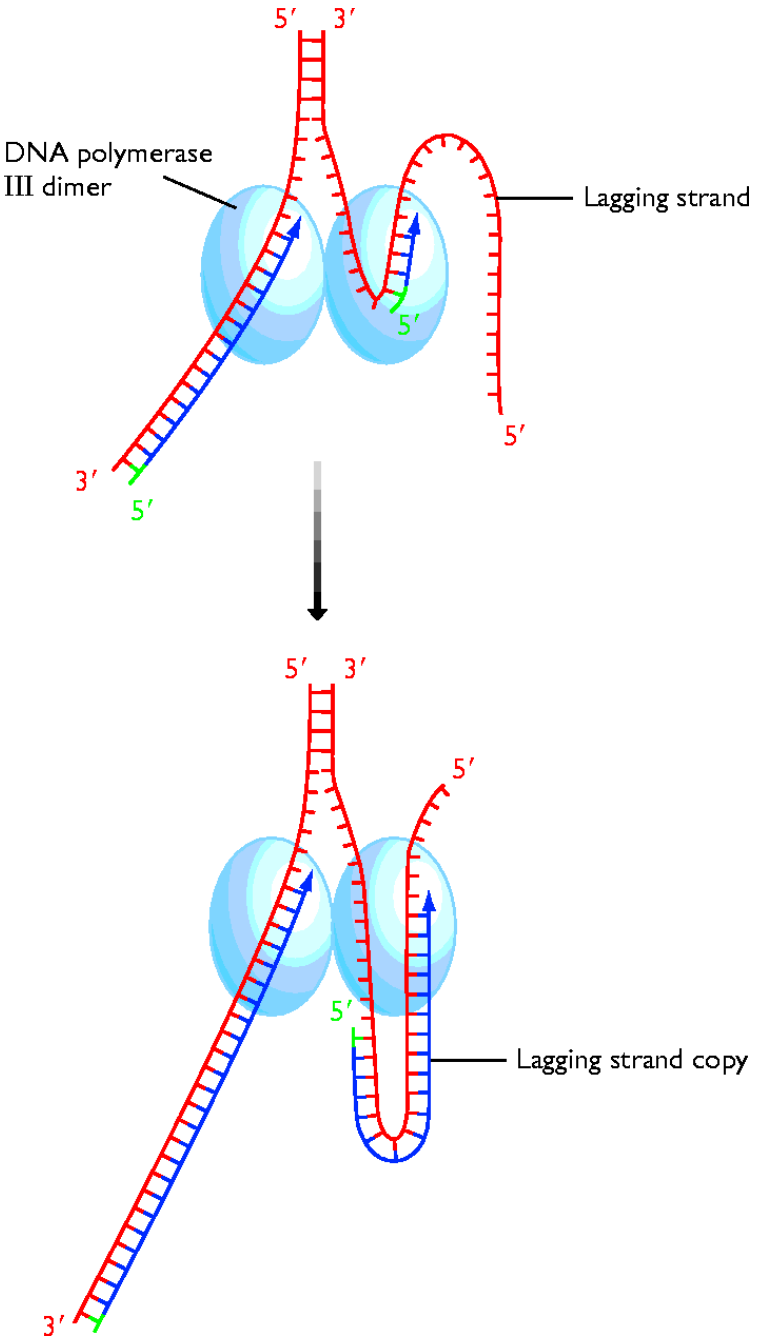
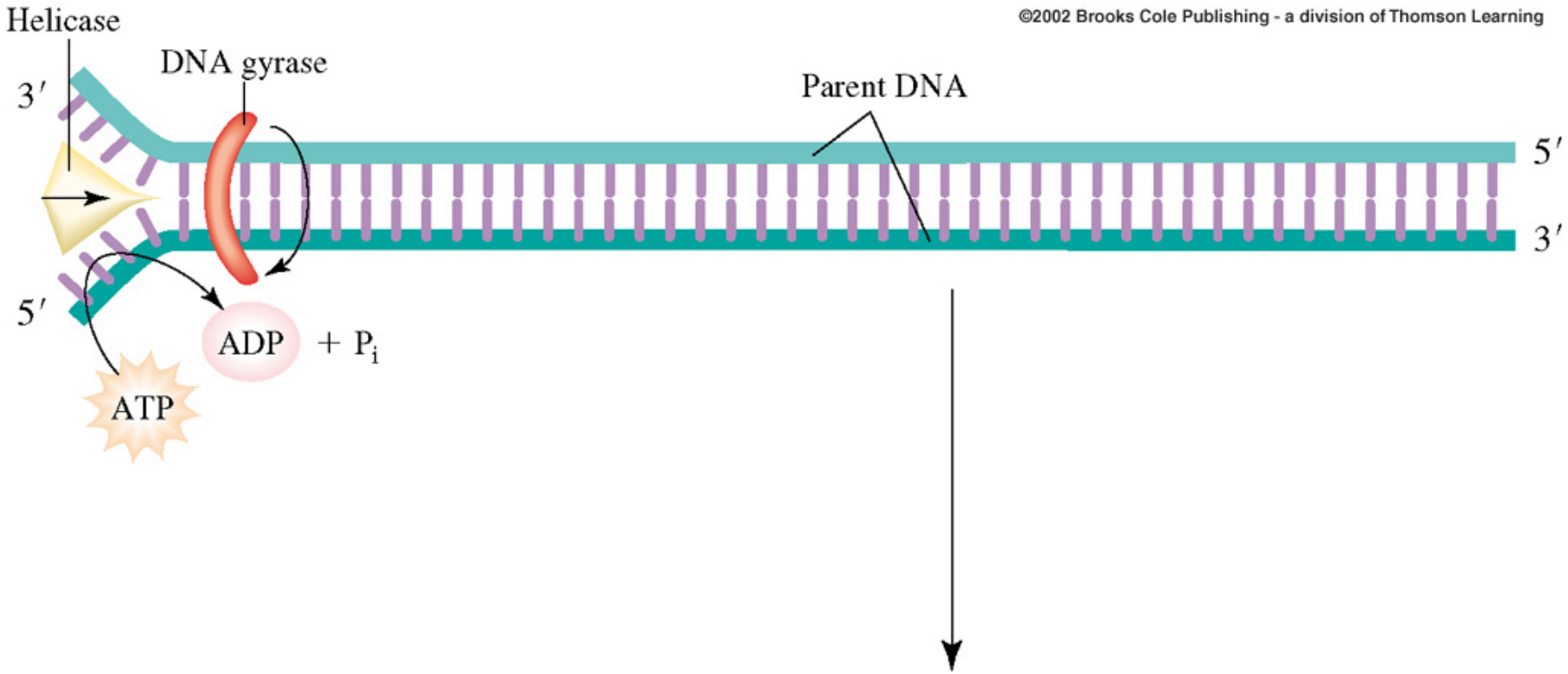


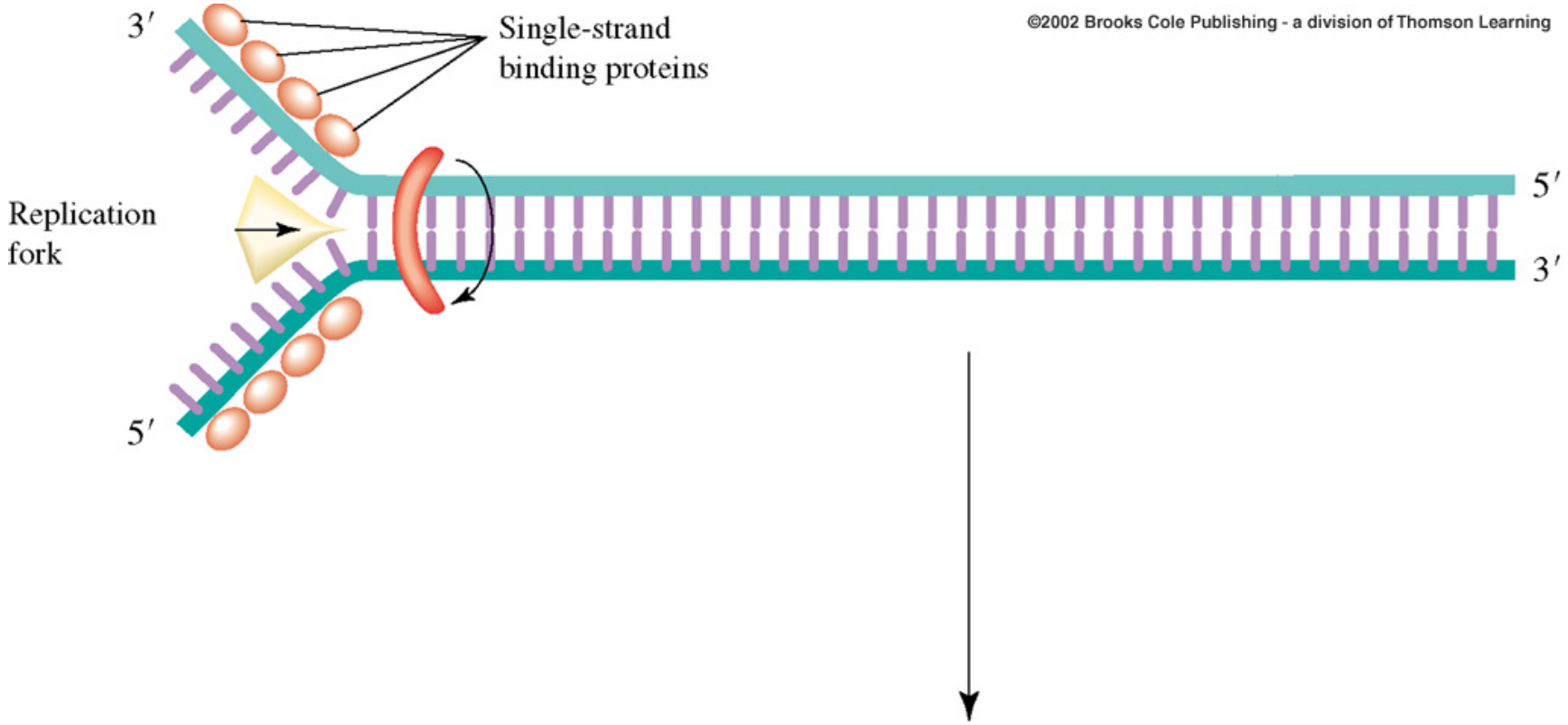
Figure 11.9a Complete scheme showing sequential steps of replication process.



1. **Helicase (unwinding protein / rep protein) recognizes and binds origin of replication.**

- **Catalyzes separation of the two DNA strands by disrupting H-bonding between base pairs.**
- **Endothermic reaction is coupled to hydrolysis of ATP.**
- **DNA gyrase (a topoisomerase) assists in unwinding by inducing supercoiling.**

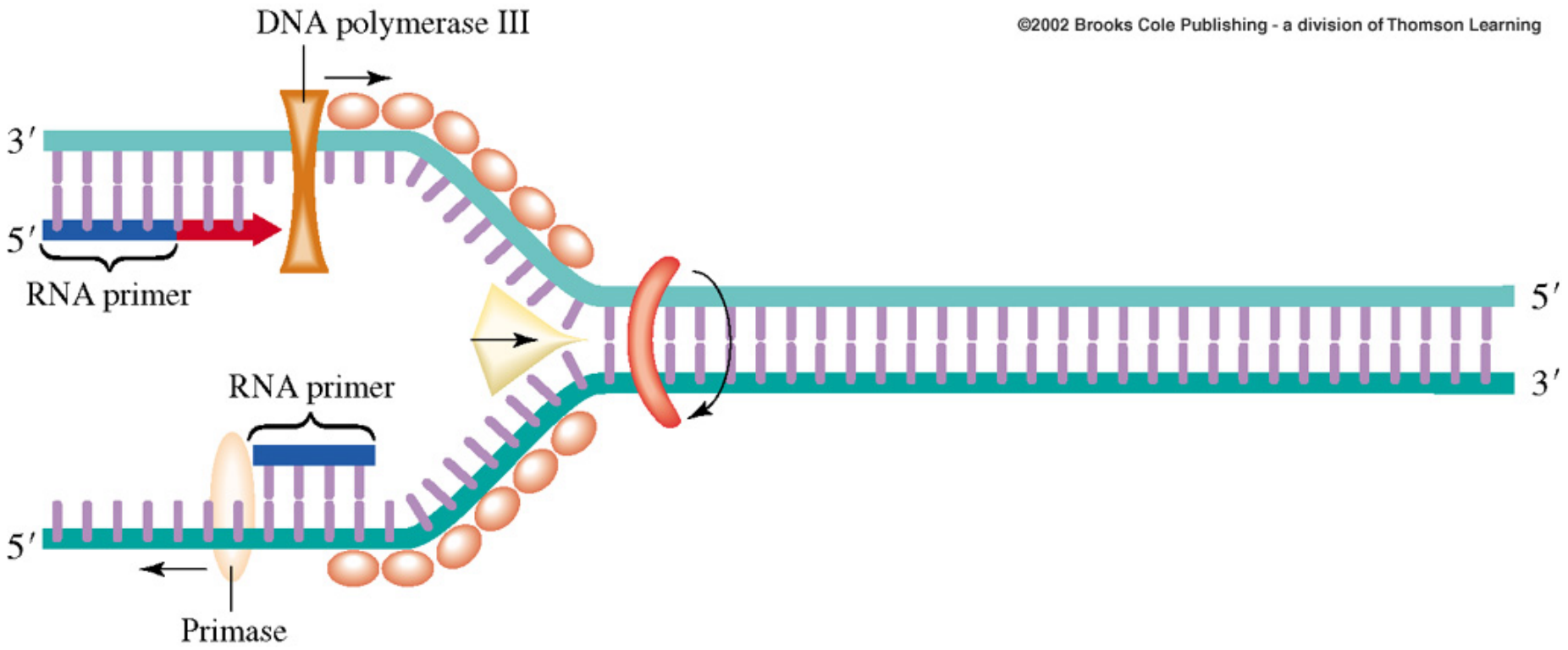
Figure 11.9b Complete scheme showing sequential steps of replication process.



2. **Single-stranded DNA binding proteins (SSB) bind exposed strands of DNA.**

- **Protect it from hydrolytic cleavage of phosphodiester bonds.**

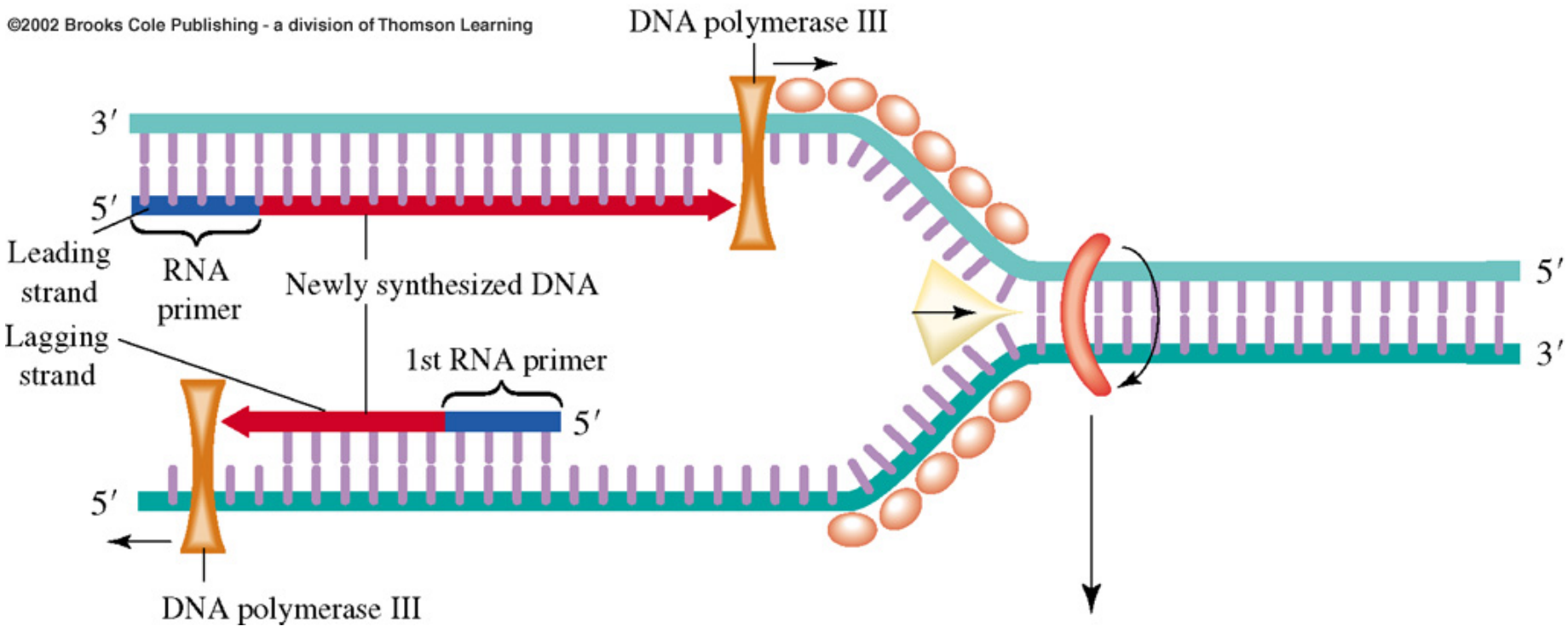
Figure 11.9c Complete scheme showing sequential steps of replication process.



3. **Primer synthesis: Short complementary stretch of RNA (4-10 bases) is synthesized by *primase* enzyme.**

- **Primer with free 3’-OH is required by DNA pol III to start 2nd strand synthesis.**
- **RNA primer is later degraded by 5’->3’ exonuclease action of DNA pol I, RNaseH enzymes.**

Figure 11.9d Complete scheme showing sequential steps of replication process.



4. **DNA synthesis by DNA pol III begins, extending leading and lagging strands.**

- **DNA synthesis continues until it meets next fragment.**

A General Model for DNA Replication

1. The DNA molecule is **unwound** and prepared for synthesis by the action of DNA gyrase, DNA helicase and the single-stranded DNA binding proteins.

2. A free 3'OH group is required for replication, but when the two chains separate no group of that nature exists. **RNA primers** are synthesized, and the free 3'OH of the primer is used to begin replication.

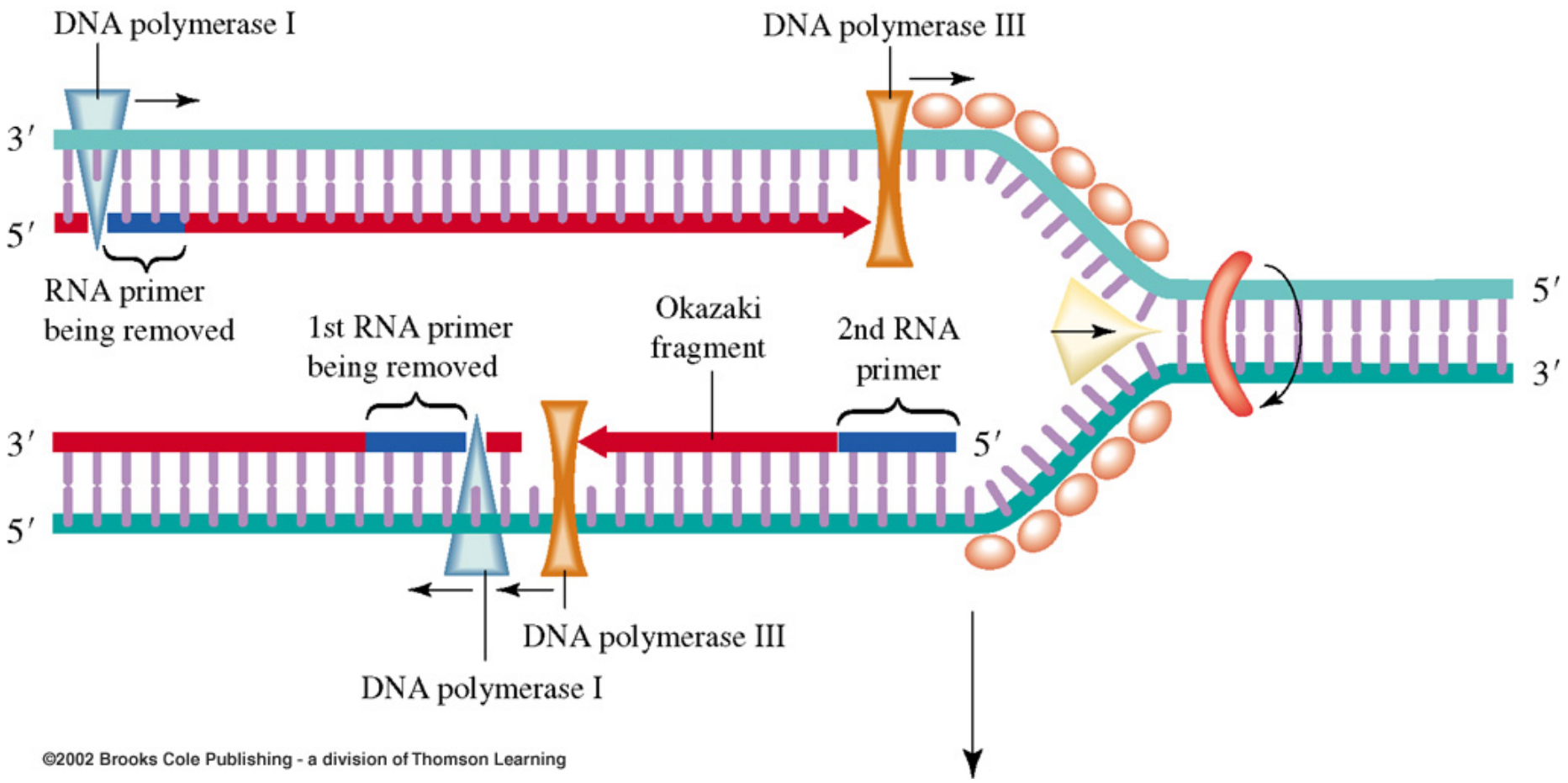
3. The replication fork moves in one direction, but DNA replication only goes in the 5' to 3' direction. This paradox is resolved by the use of Okazaki fragments. These are short, discontinuous replication products that are produced off the **lagging strand**. This is in comparison to the continuous strand that is made off the **leading strand**.

4. The final product does not have RNA stretches in it. These are removed by the 5' to 3' exonuclease action of Polymerase I.

5. The final product does not have any gaps in the DNA that result from the removal of the RNA primer. These are filled in by the 5' to 3' polymerase action of DNA Polymerase I.

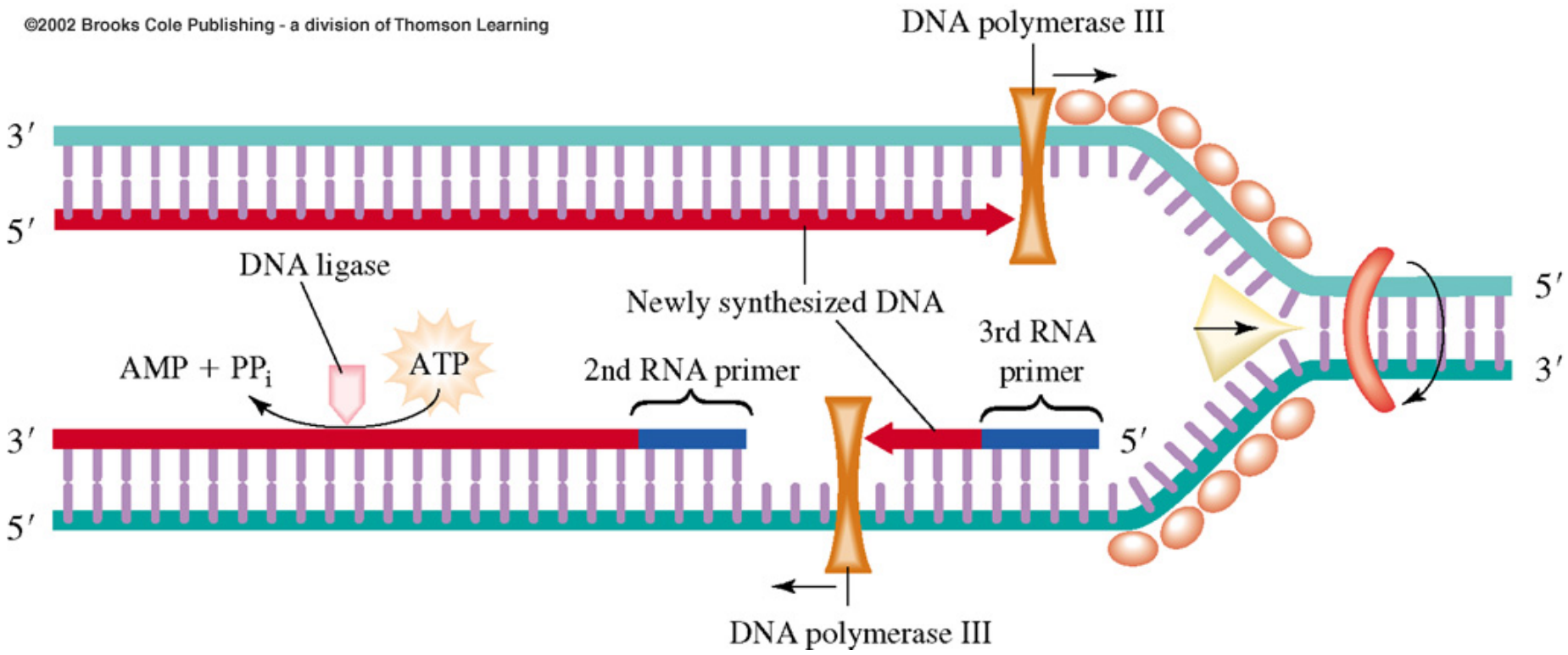
6. DNA polymerase does not have the ability to form the final bond. This is done by the enzyme DNA **ligase**.

Figure 11.9e Complete scheme showing sequential steps of replication process.



5. **RNA primers are removed by 5’→3’ exonuclease action of DNA polymerase I, small gaps are filled in by DNA polymerase I.**

Figure 11.9f Complete scheme showing sequential steps of replication process.



6. **Final gap between new strands is closed by DNA ligase enzyme.**

- **Requires ATP to join 3’ OH on one fragment and 5’ phosphate on second fragment.**

A General Model for DNA Replication

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2. A free 3'OH group is required for replication, but when the two chains separate no group of that nature exists. **RNA primers** are synthesized, and the free 3'OH of the primer is used to begin replication.

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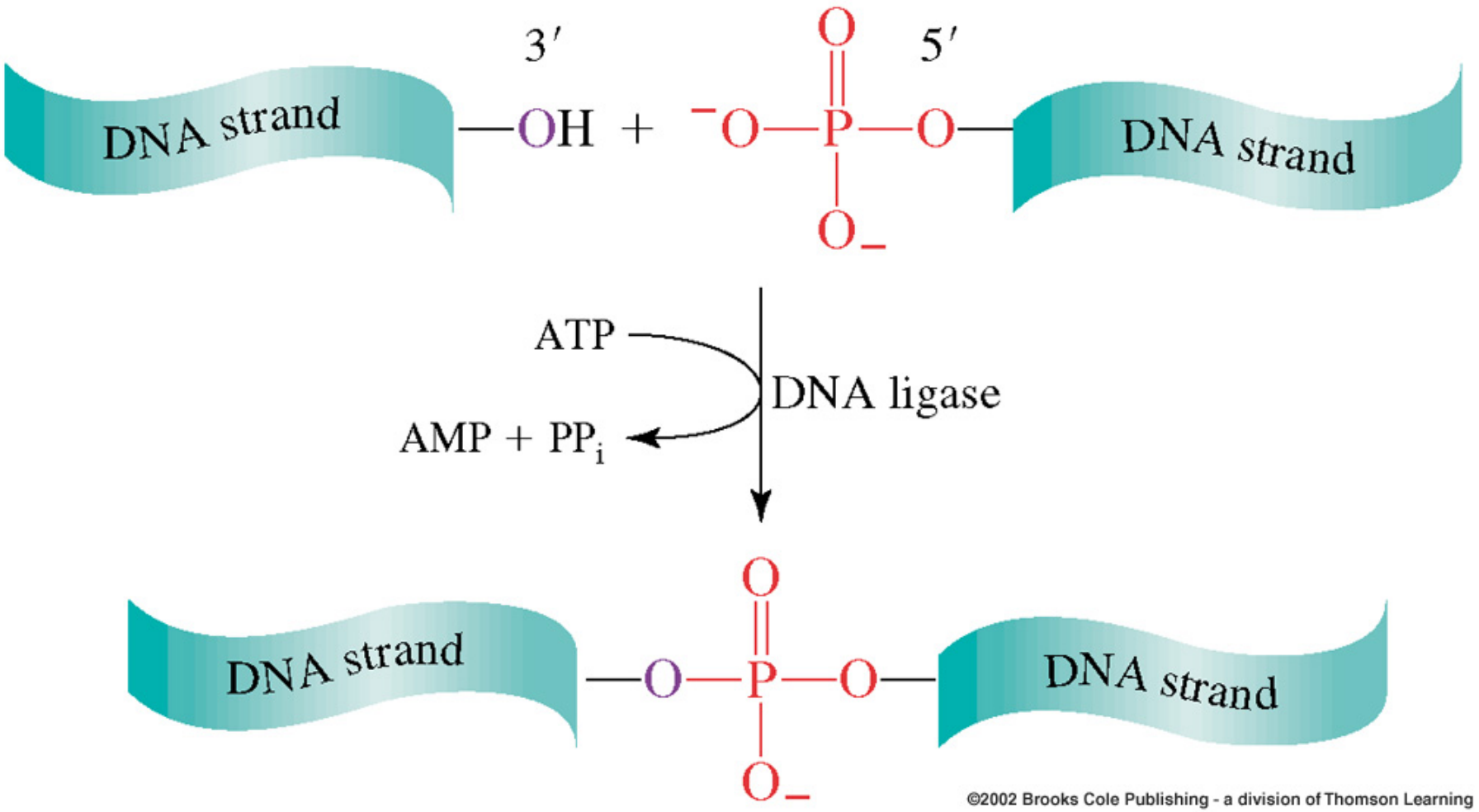
6. DNA polymerase does not have the ability to form the final bond. This is done by the enzyme DNA ligase.

Figure 11.10 The DNA ligase-catalyzed reaction to close the final phosphodiester bond in newly synthesized DNA.

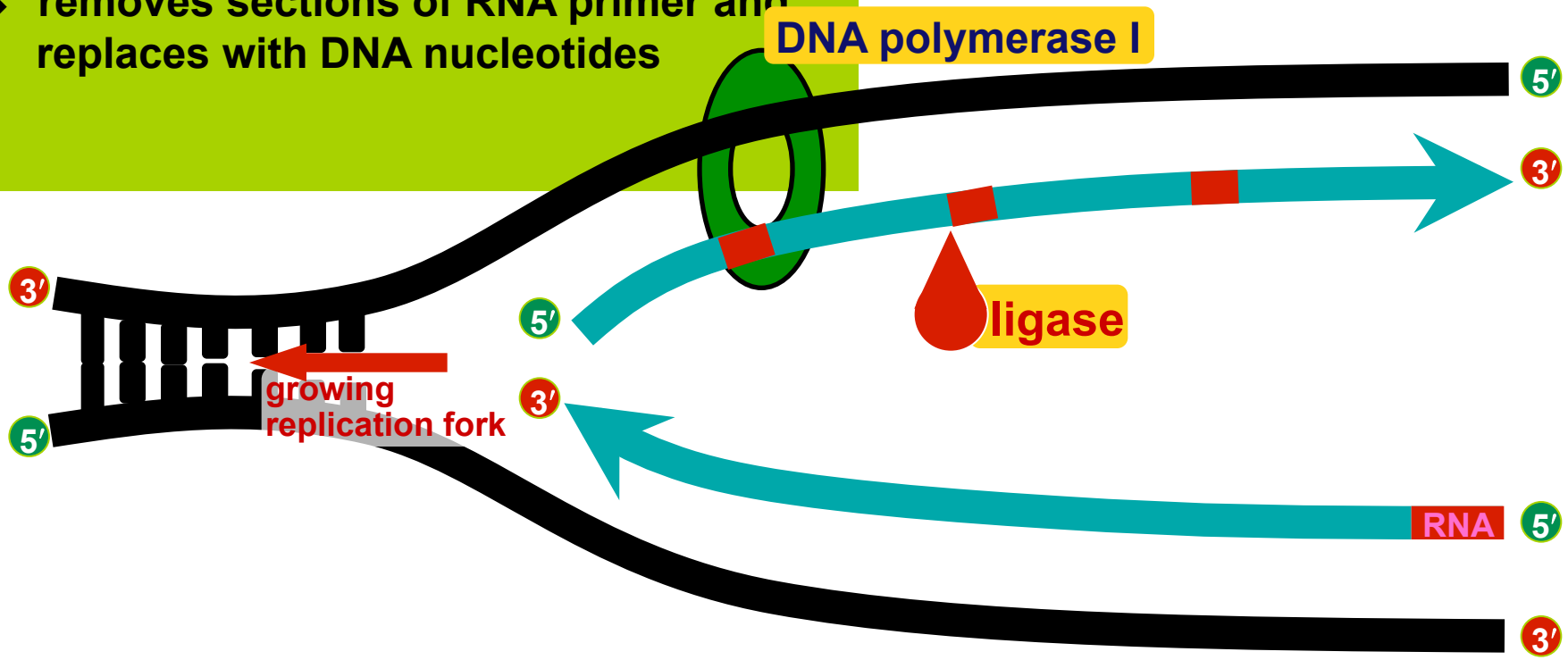


ATP is required as a source of energy for this endergonic reaction.

Replacing RNA primers with DNA

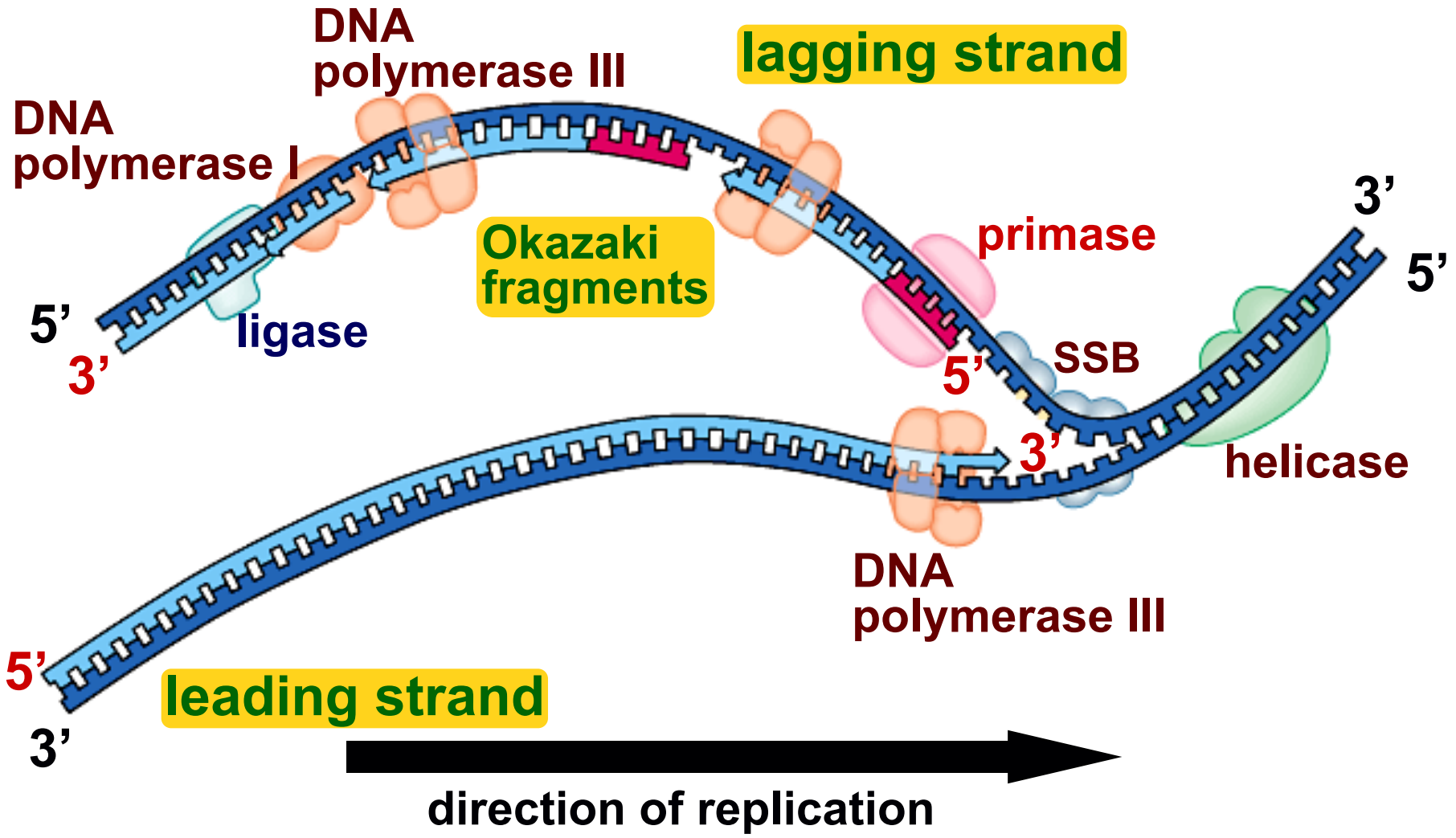
DNA polymerase I

- **removes sections of RNA primer and replaces with DNA nucleotides**



But DNA polymerase I still can only build onto 3′ end of an existing DNA strand

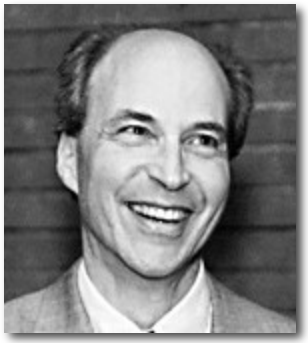
Replication fork



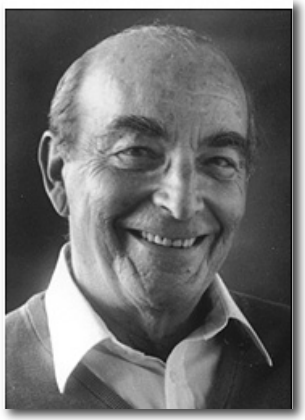
SSB = single-stranded binding proteins

DNA polymerases

- DNA polymerase III
  - 1000 bases/second*!*
  - main DNA builder
- DNA polymerase I
  - 20 bases/second
  - editing, repair & primer removal

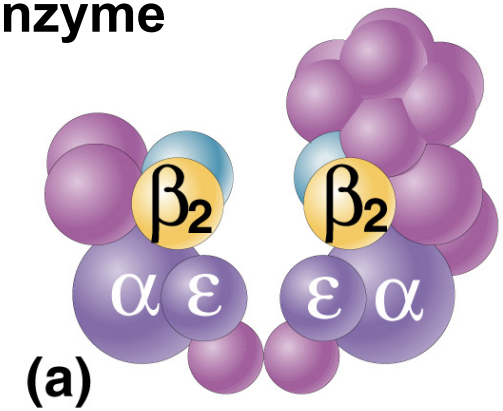


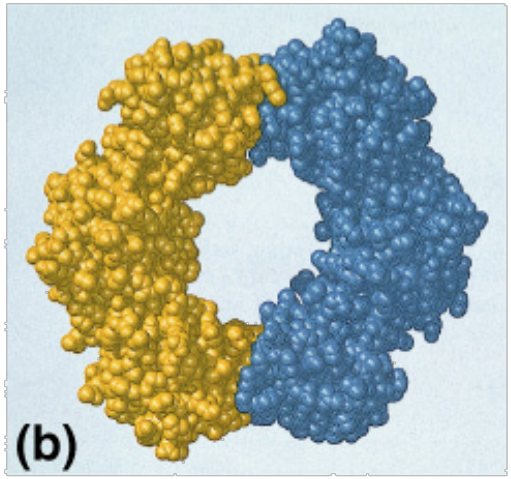
Roger Kornberg
2006

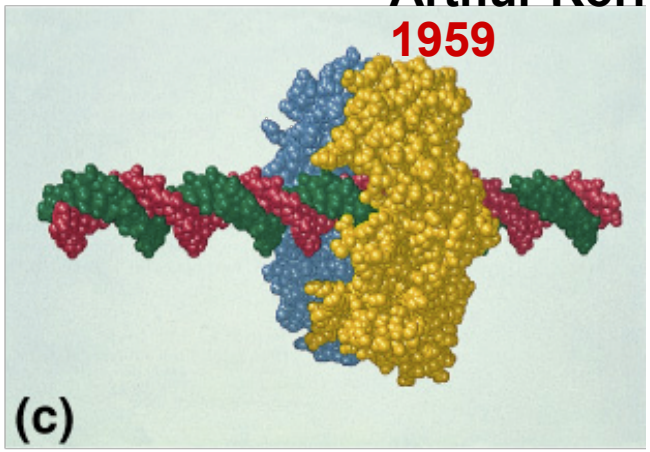


Arthur Kornberg
1959

DNA polymerase III enzyme

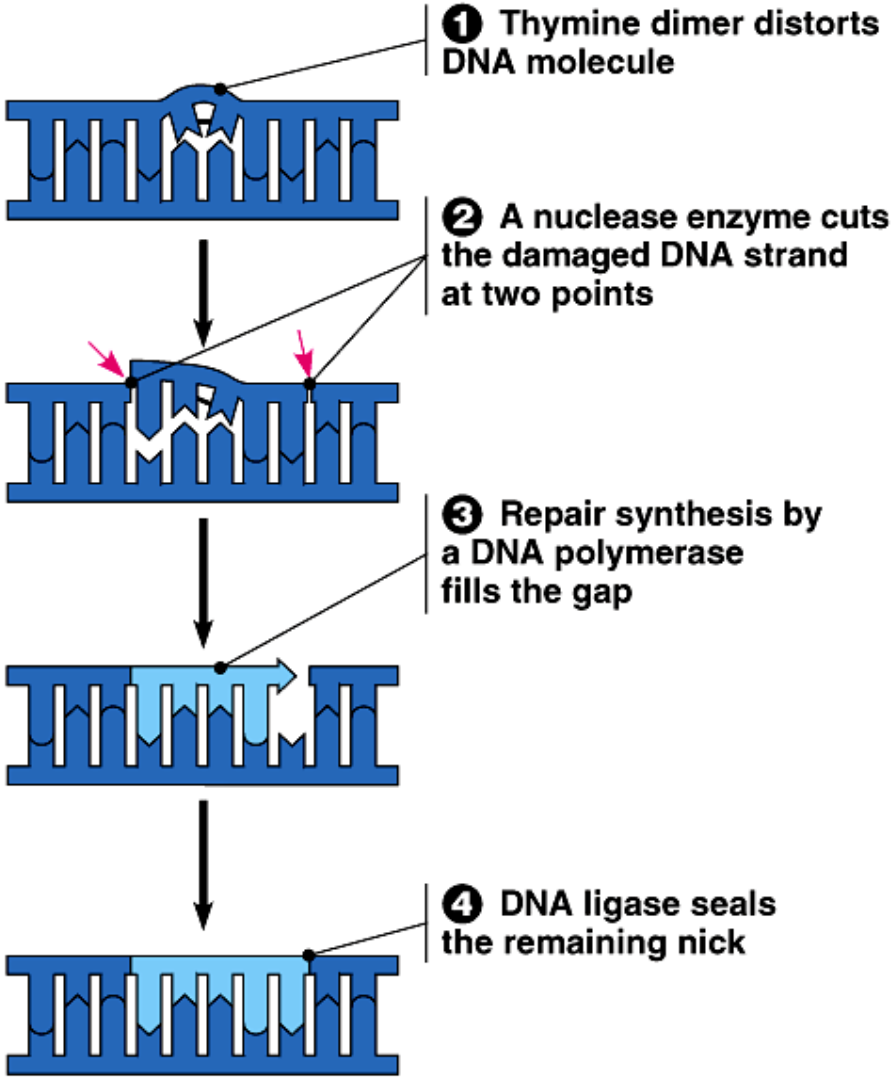






Editing & proofreading DNA

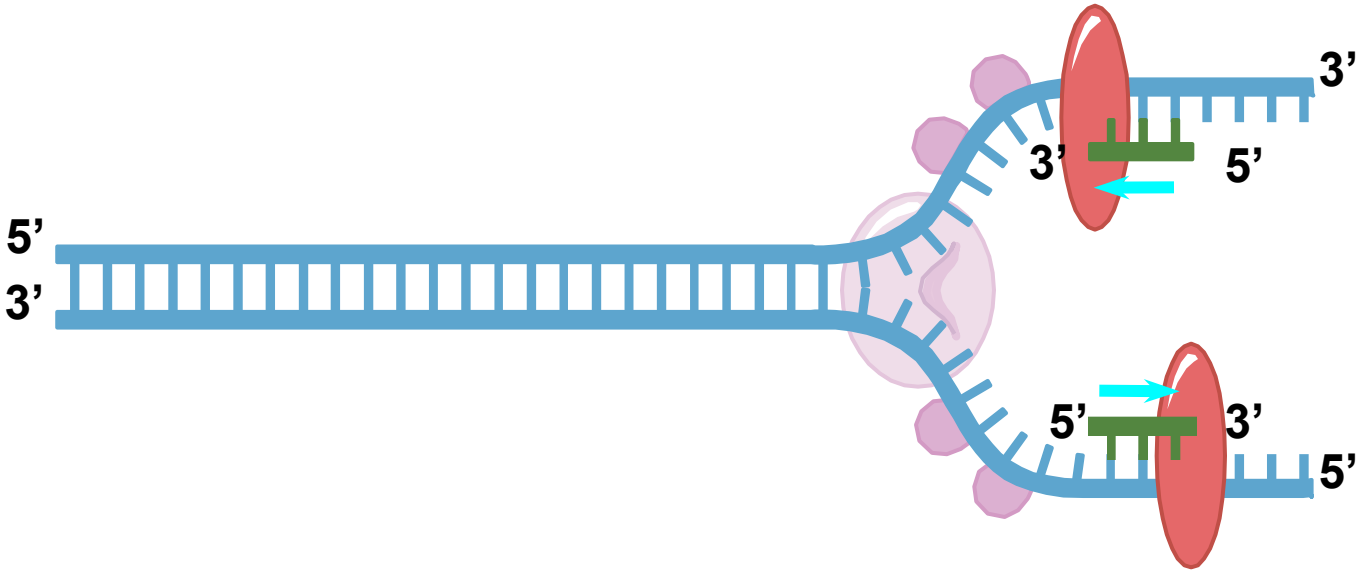
- 1000 bases/second = lots of typos!
- DNA polymerase I
  - proofreads & corrects typos
  - repairs mismatched bases
  - removes abnormal bases
    - repairs damage throughout life
  - reduces error rate from 1 in 10,000 to 1 in 100 million bases



Fast & accurate!

- It takes *E. coli* <1 hour to copy 5 million base pairs in its single chromosome
  - divide to form 2 identical daughter cells
- Human cell copies its 6 billion bases & divide into daughter cells in only few hours
  - remarkably accurate
  - only ~1 error per 100 million bases
  - ~30 errors per cell cycle

Replication

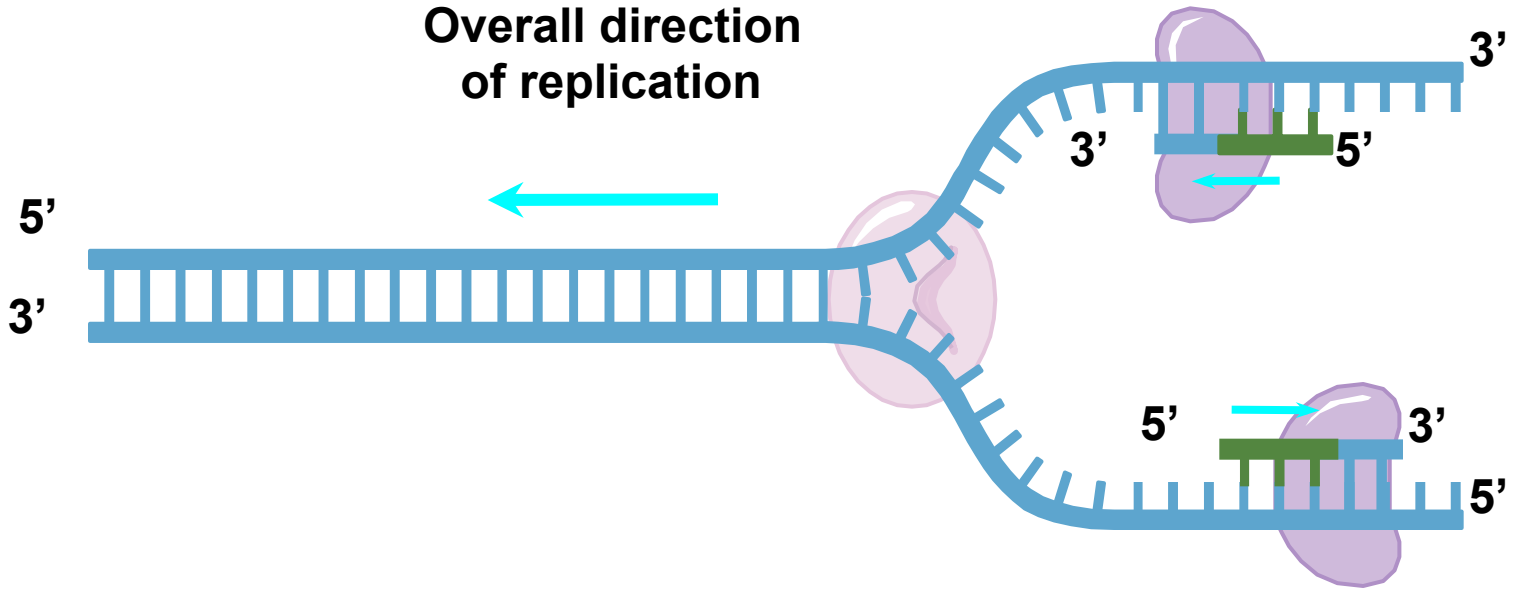


Helicase protein binds to DNA sequences called origins and unwinds DNA strands.

Binding proteins prevent single strands from rewinding.

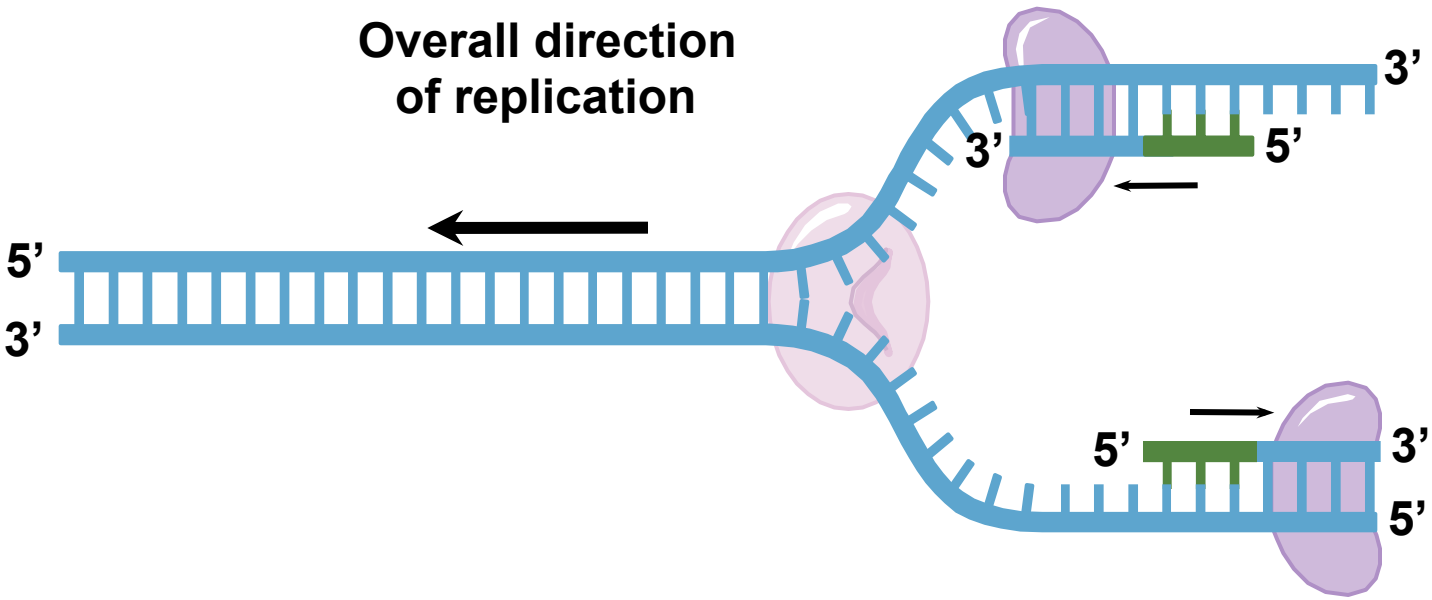
Primase protein makes a short segment of RNA complementary to the DNA, a primer.

Replication



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

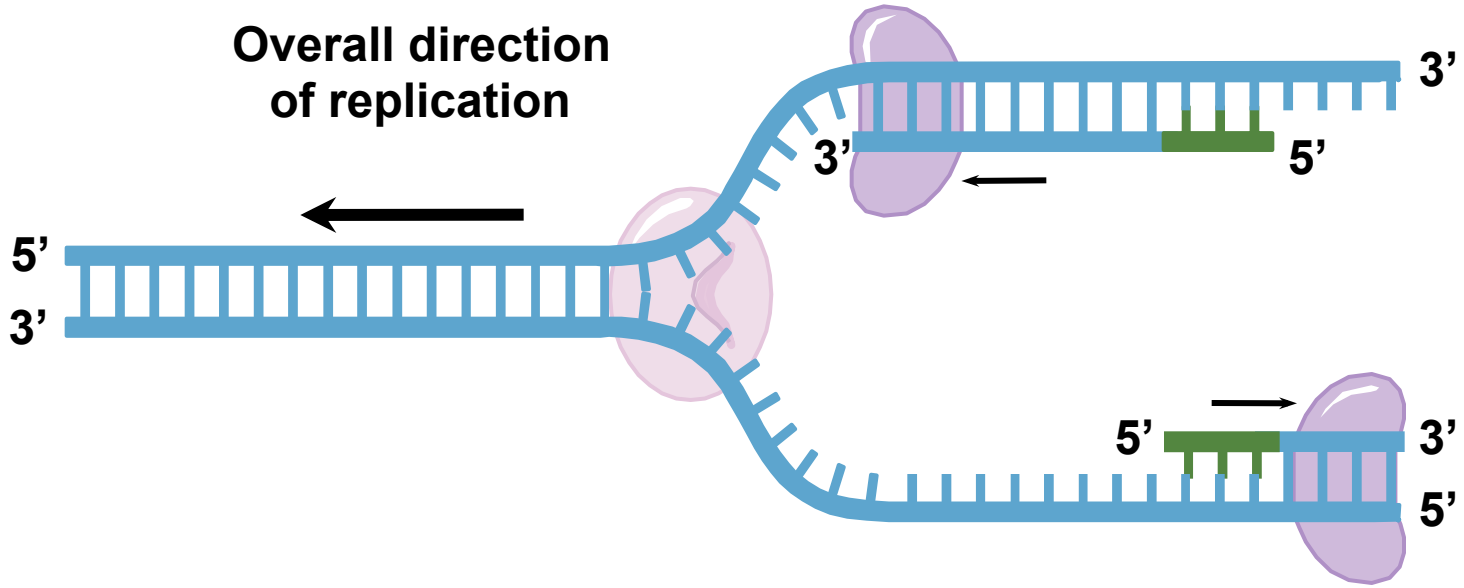
Replication



DNA polymerase enzyme adds DNA nucleotides to the RNA primer.

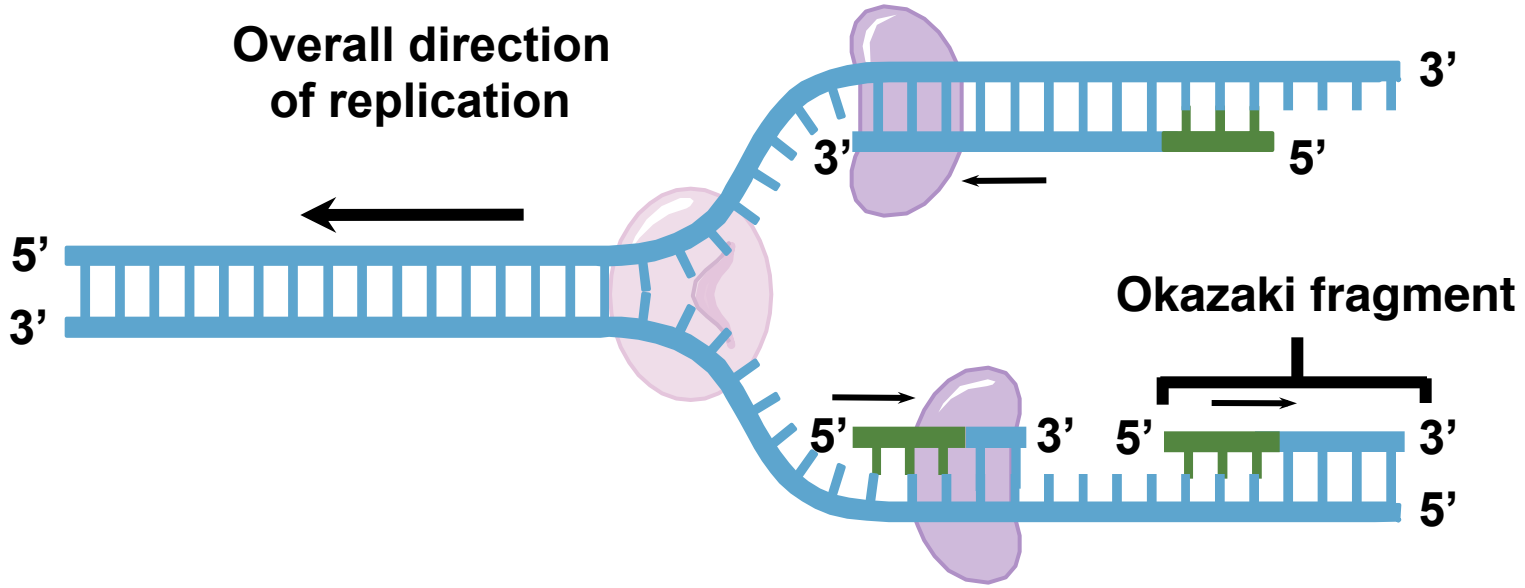
DNA polymerase proofreads bases added and replaces incorrect nucleotides.

Replication



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

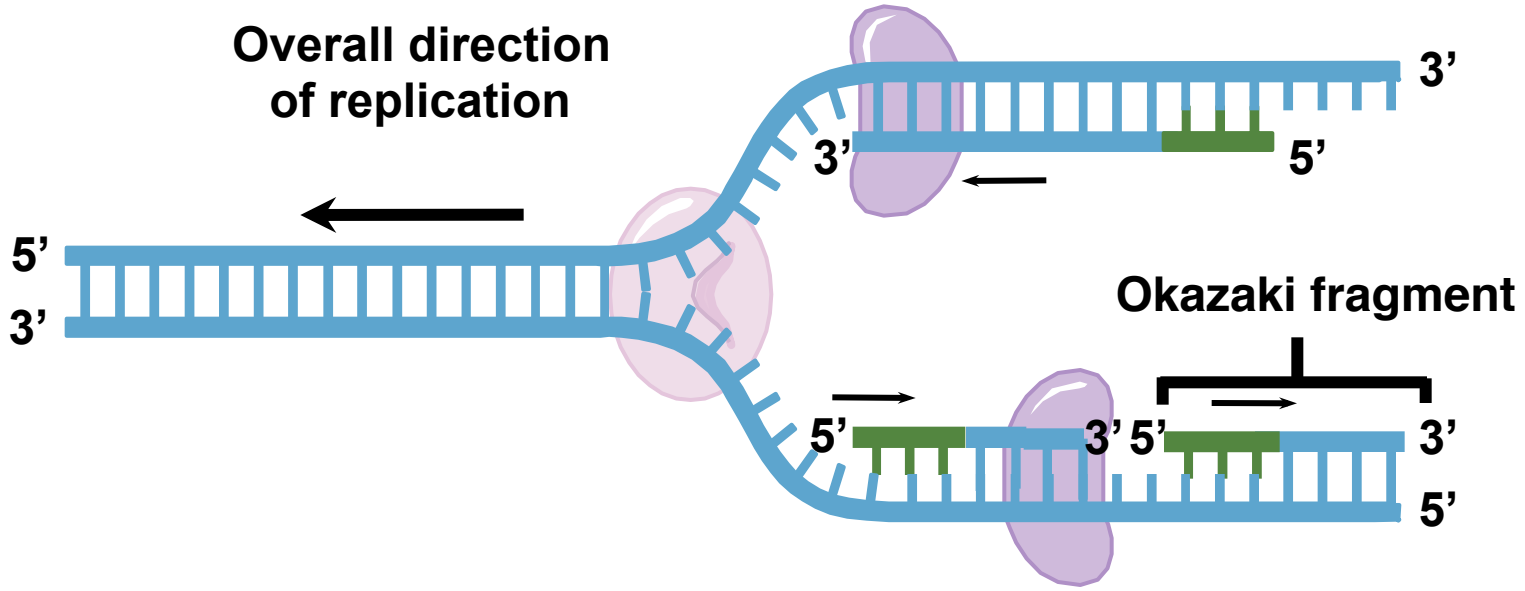
Replication



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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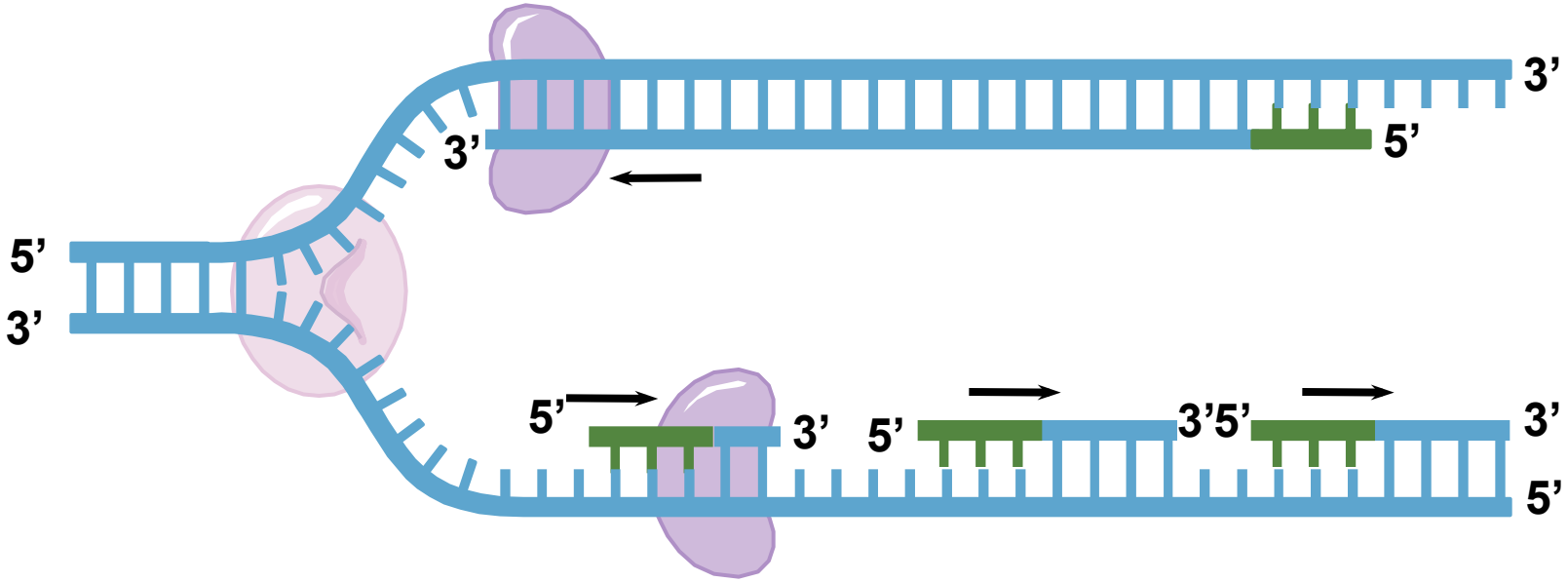

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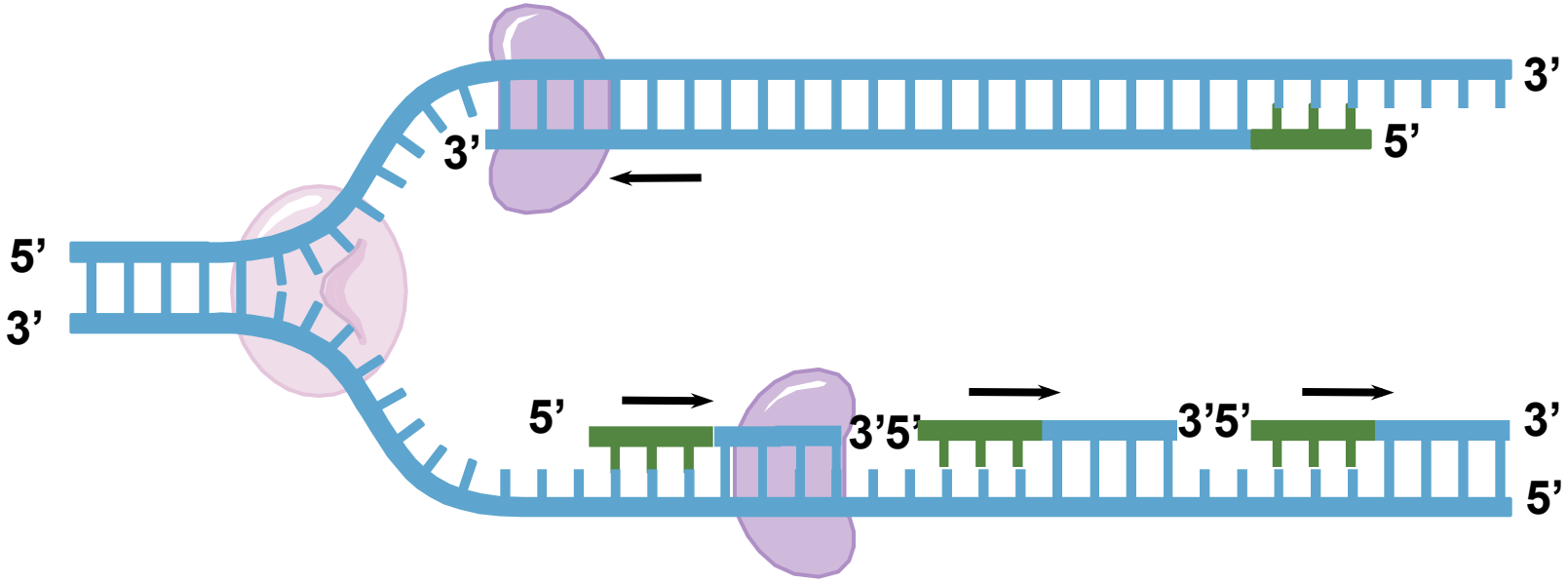

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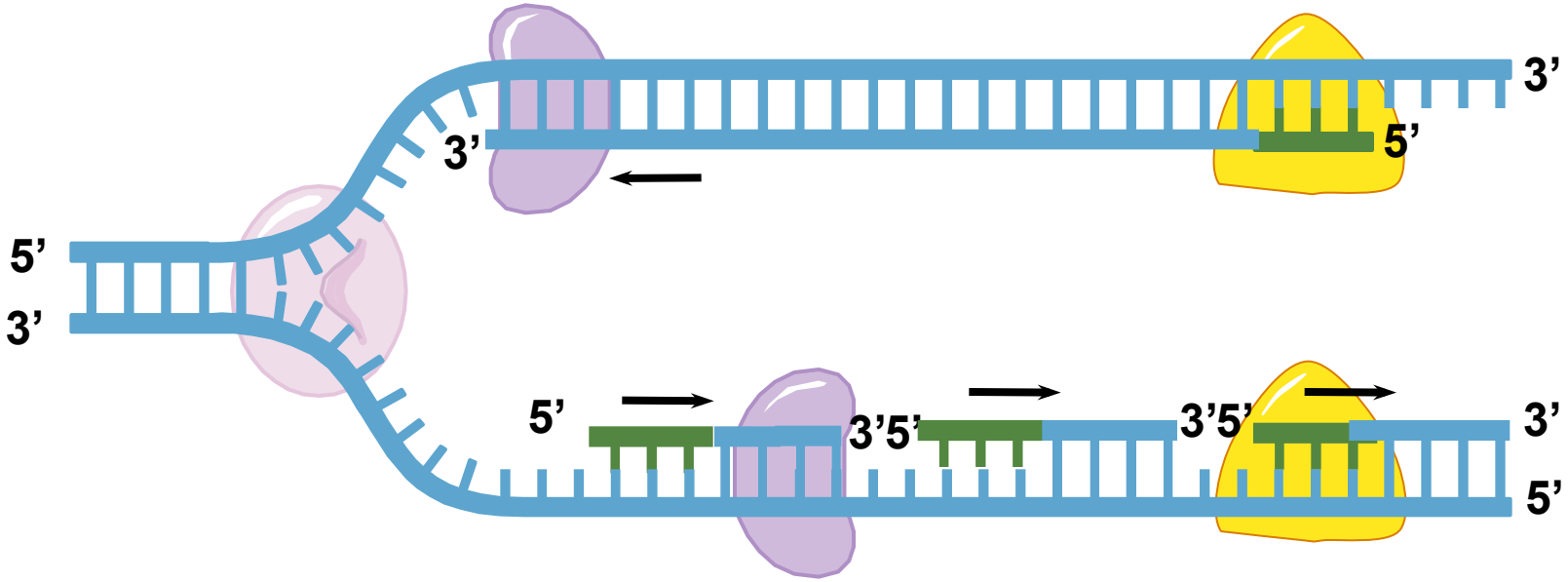
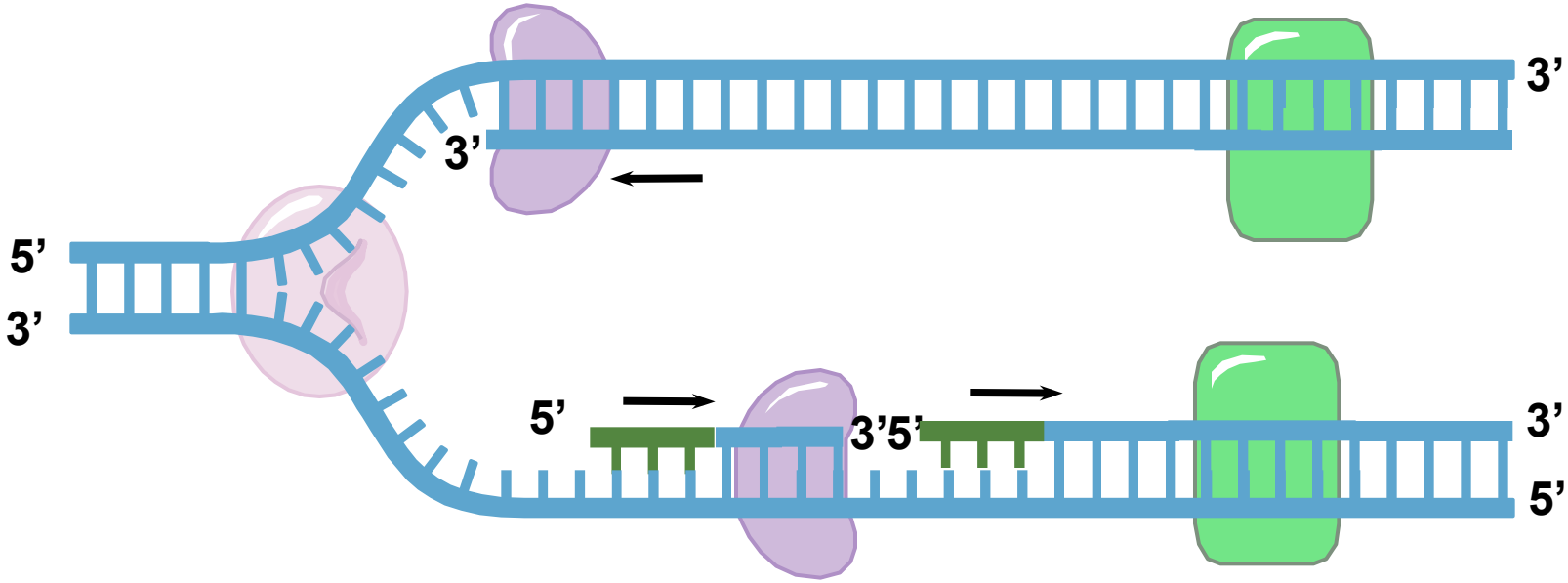
Replication



Exonuclease activity of DNA polymerase I removes RNA primers.

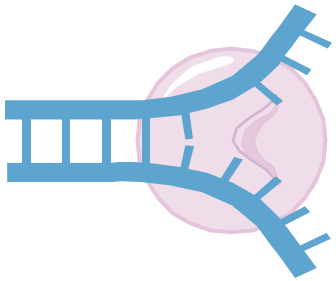
Replication



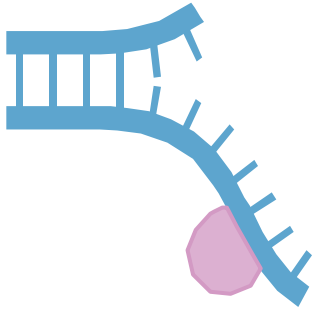
Polymerase activity of DNA polymerase I fills the gaps.

Ligase forms bonds between sugar-phosphate backbone.

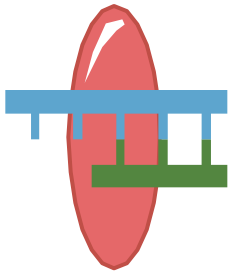
Enzymes in DNA replication



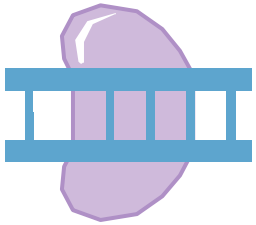
Helicase unwinds parental double helix



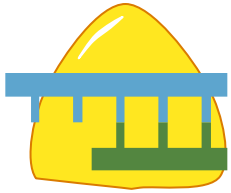
Binding proteins stabilise separate strands



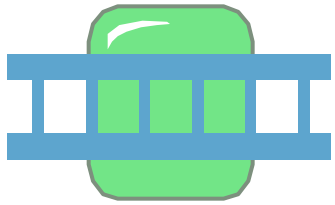
Primase adds short primer to template strand



DNA polymerase III binds nucleotides to form new strands



DNA polymerase I (Exonuclease) removes RNA primer and inserts the correct bases



Ligase joins Okazaki fragments and seals other nicks in sugar-phosphate backbone

Collaboration of Proteins at the Replication Fork