

# Lecture 5

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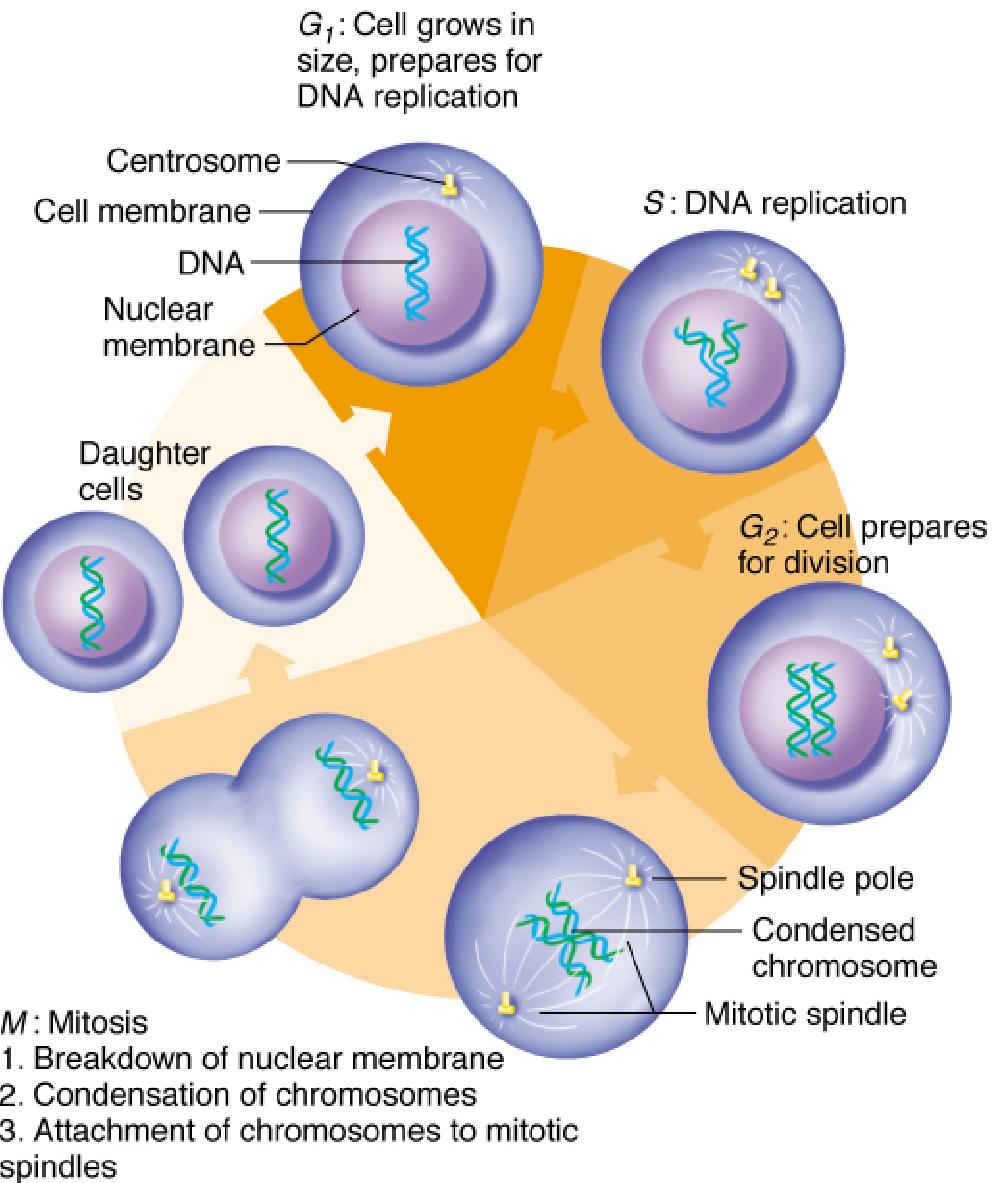
## DNA Replication

April 12, 2016  
Pyle

# The Eukaryotic Cell Cycle

S phase or DNA replication

Replication is  
carefully  
Regulated by the  
timing  
of the Cell cycle  
and critical cell cycle  
“checkpoints.”



# DNA Replication (Overview)

## 1. Semi-conservative replication

how to test Watson-Crick hypothesis?

Meselson-Stahl experiment (1958)

## 2. Replication process

Replication origin and supercoil problem

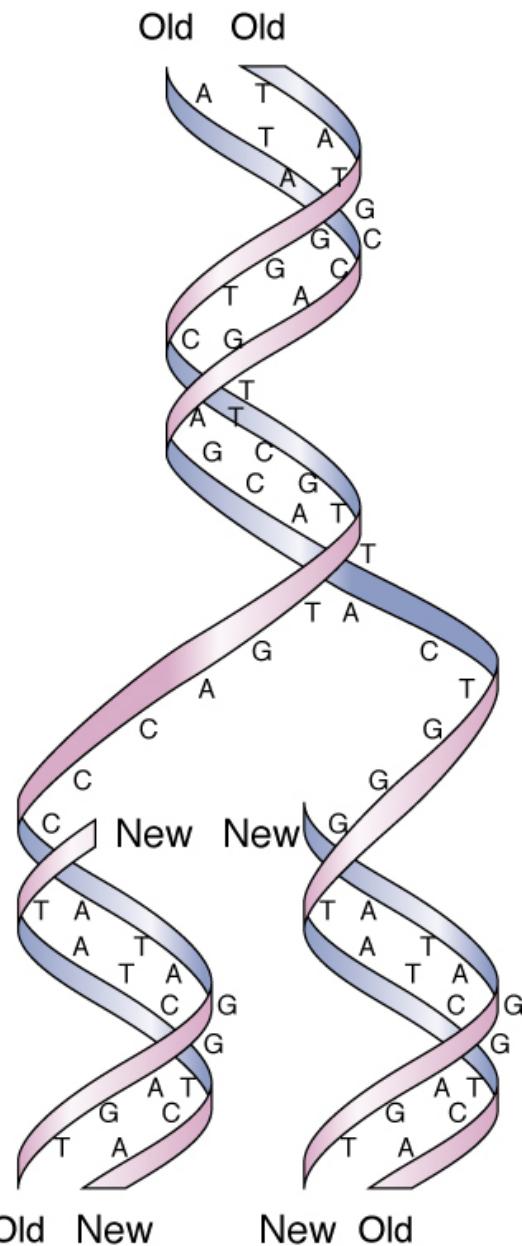
Replication fork and bi-directional replication

Leading strand vs lagging strand

## 3. DNA replication enzymes

## 4. Proof reading of DNA replication

# Watson-Crick semiconservative hypothesis



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# Difference between conservative and semiconservative DNA replication

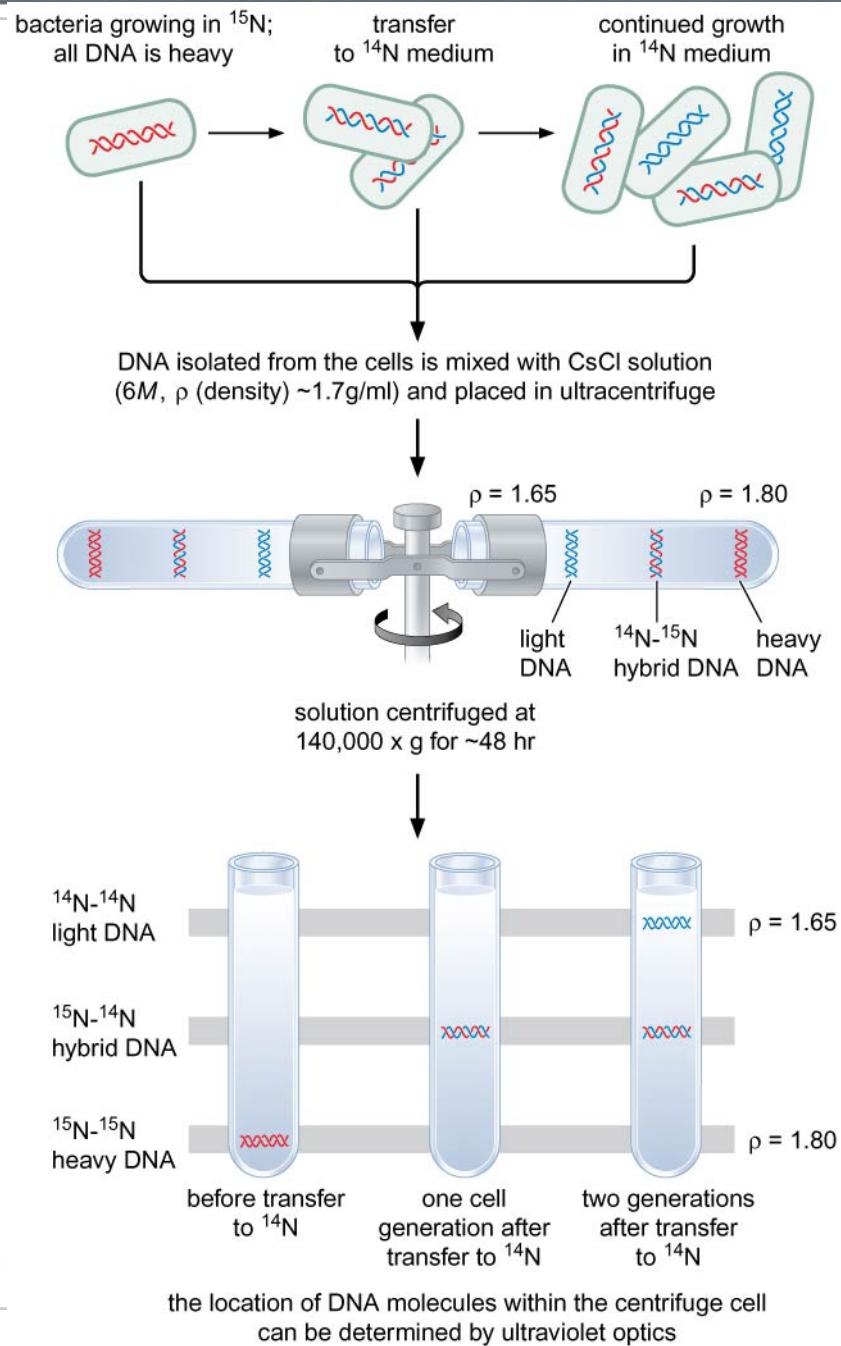
parent

**Conservative:**  
Both parental DNA  
strands are  
conserved in the  
daughter DNA

**Semi-conservative:**  
Only one of the two  
parental strands is  
conserved in the  
daughter dsDNA

daughter





## DNA replication is Semi-Conservative

Figure 2-9- Use of CsCl to demonstrate separation of complementary strands p29 of Watson 7<sup>th</sup> ed

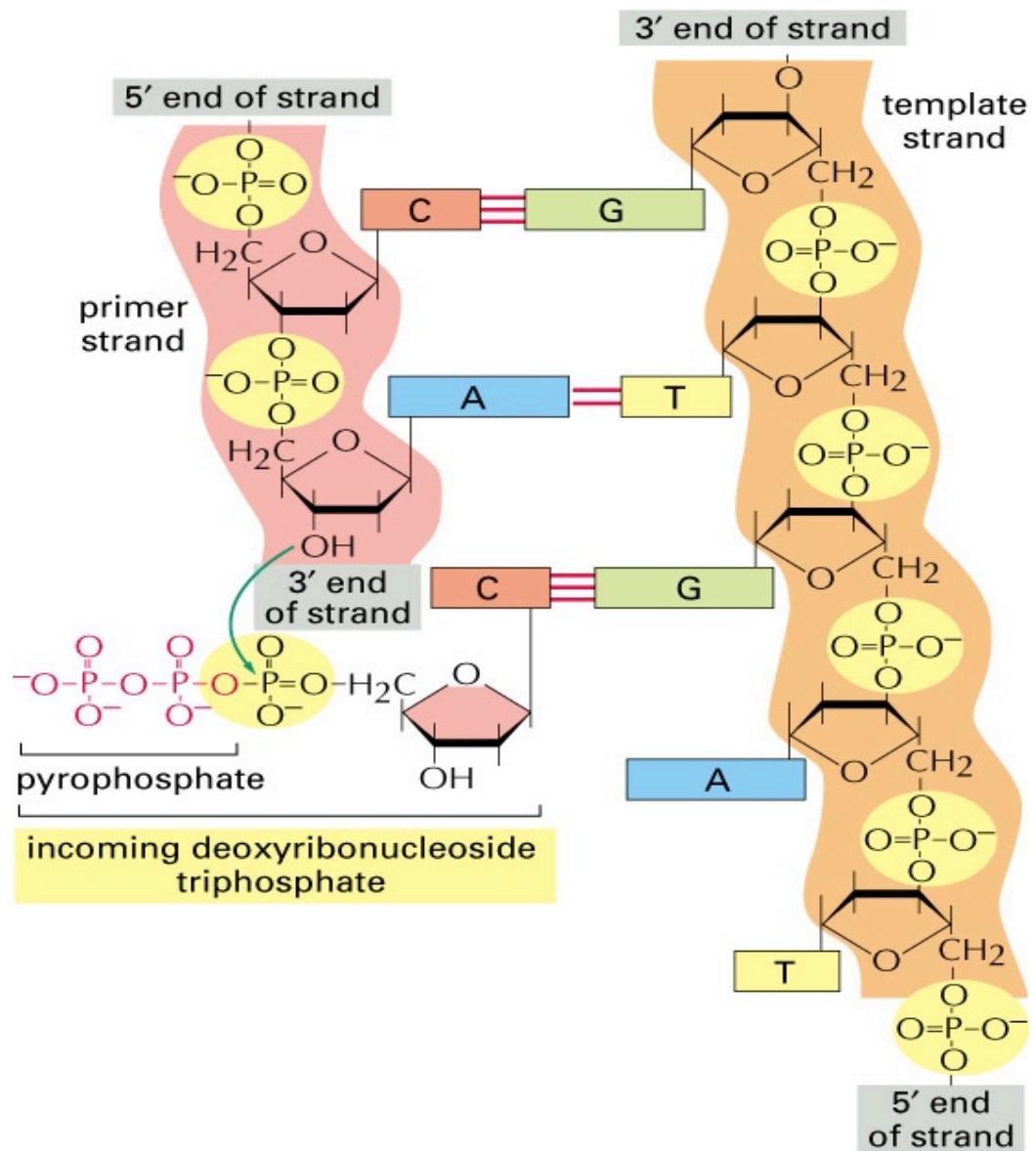
## Overview of DNA replication movie

<https://www.hhmi.org/bioInteractive/dna-replication-basic-detail>

# **Features of DNA Replication**

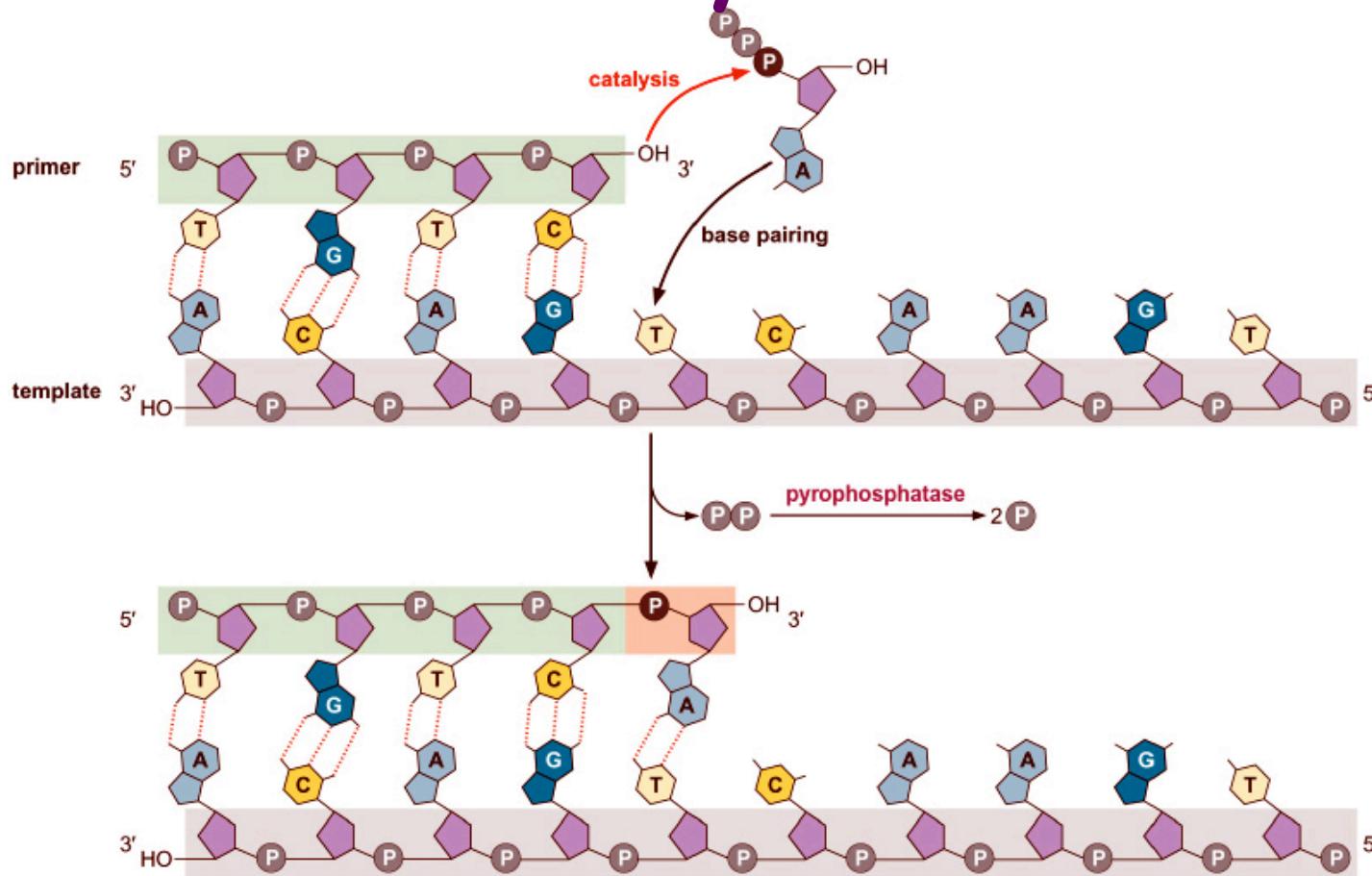
- 1. DNA replication starts from a replication origin**
- 2. DNA replication is a DNA template-dependent polymerization process that needs RNA primers**
- 3. DNA synthesis is always from 5' to 3', so replication of a dsDNA has to proceed in opposite directions (bidirectional replication)**
- 4. DNA is synthesized continuously (leading strand), as well as discontinuously (lagging strand-synthesized as Okazaki fragments)**
- 5. DNA replication is very accurate and fast**

# Direction of DNA Synthesis **5' to 3'**



**The 3' hydroxyl group of the existing DNA is where the incoming nucleotide will be added.**

# DNA synthesis



Can be viewed as the extension of existing DNA (or RNA primer) through base pairing with nucleotides on the template.

Substrates: Deoxyribonucleoside triphosphates

Chemistry: 3' OH attacks phosphate on incoming nucleoside triphosphate

# Origin of replication

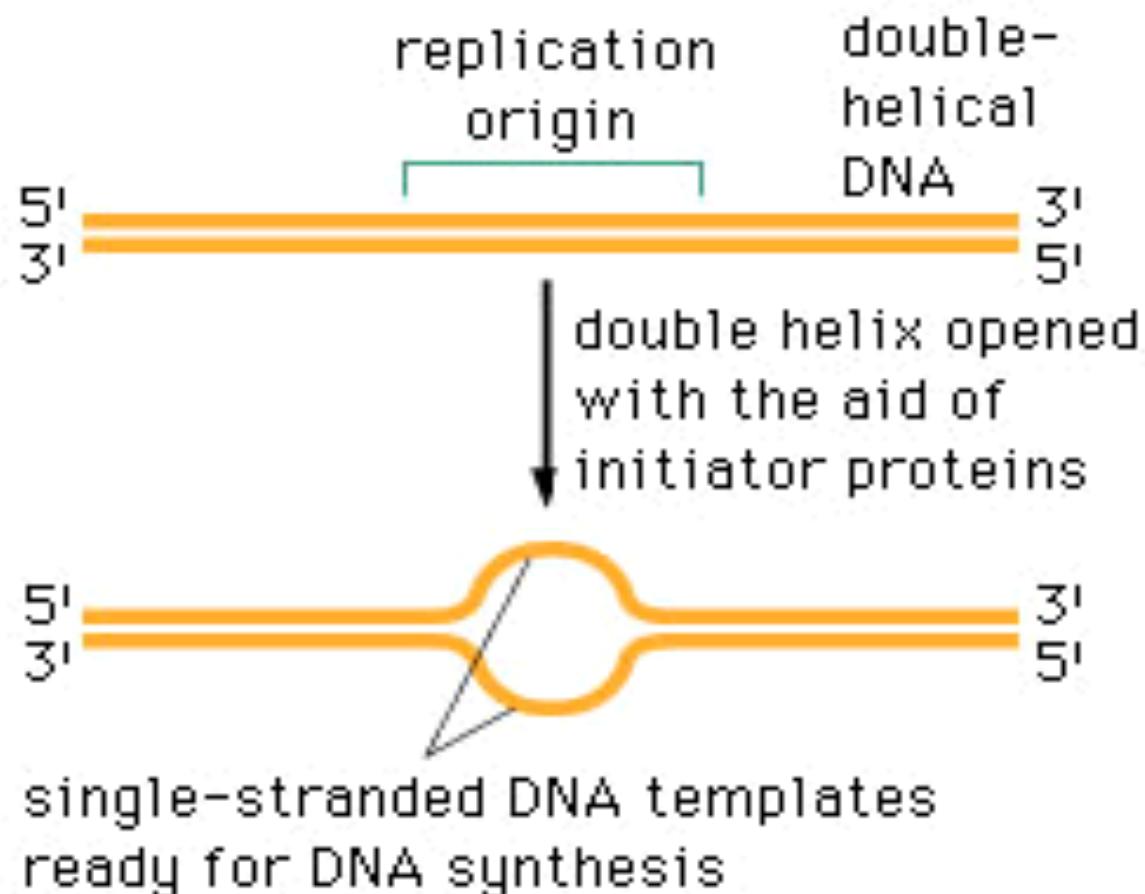
**In a large stretch of genomic DNA, where will replication begin?**

The replication origin is a specific place in the genome where dsDNA is first opened to allow for replication

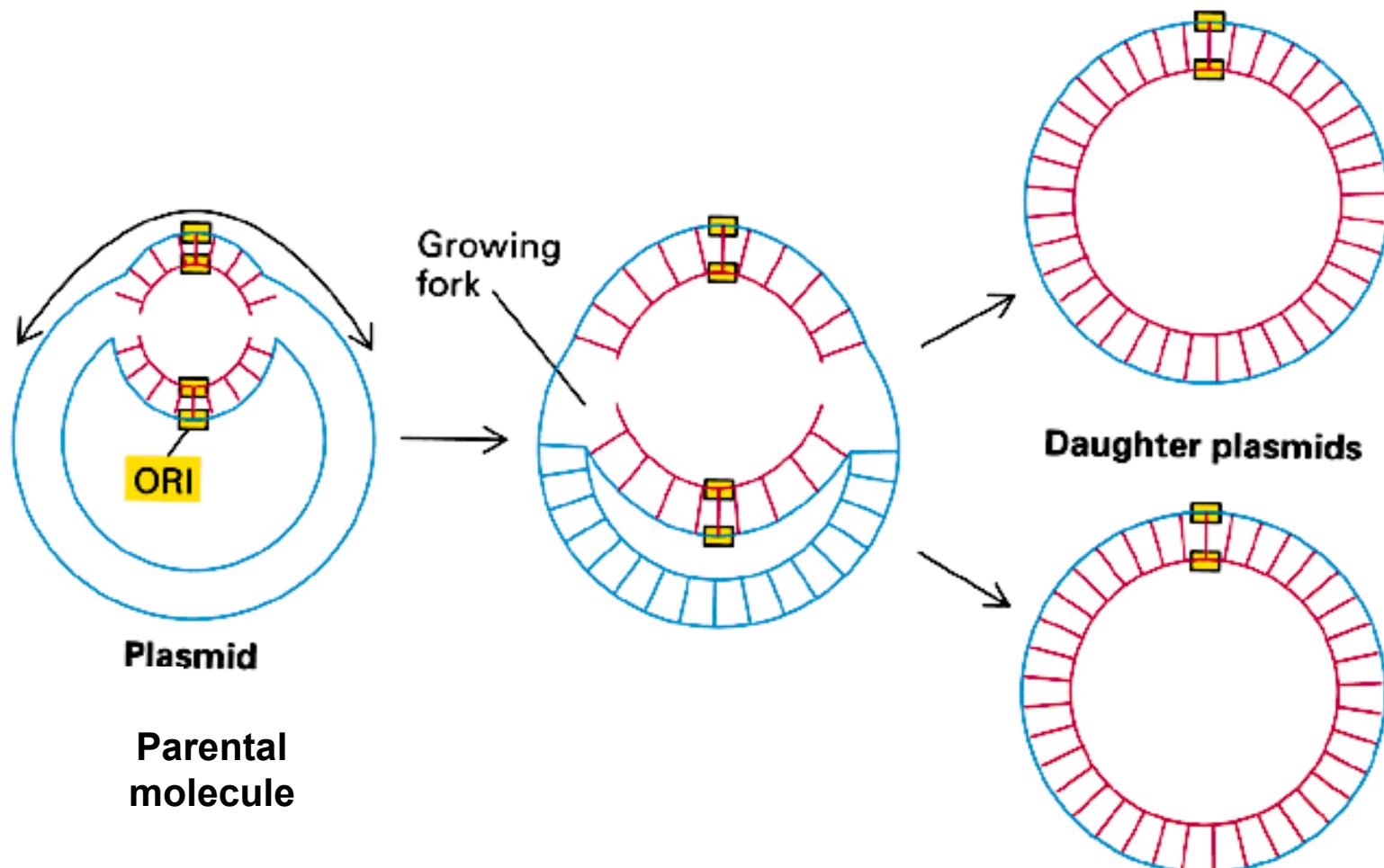
**Prokaryote**—There is one single origin per genome (or plasmid), also called a replicon

**Eukaryote**—have multiple origins in each chromosome (~10,000 in the human genome)

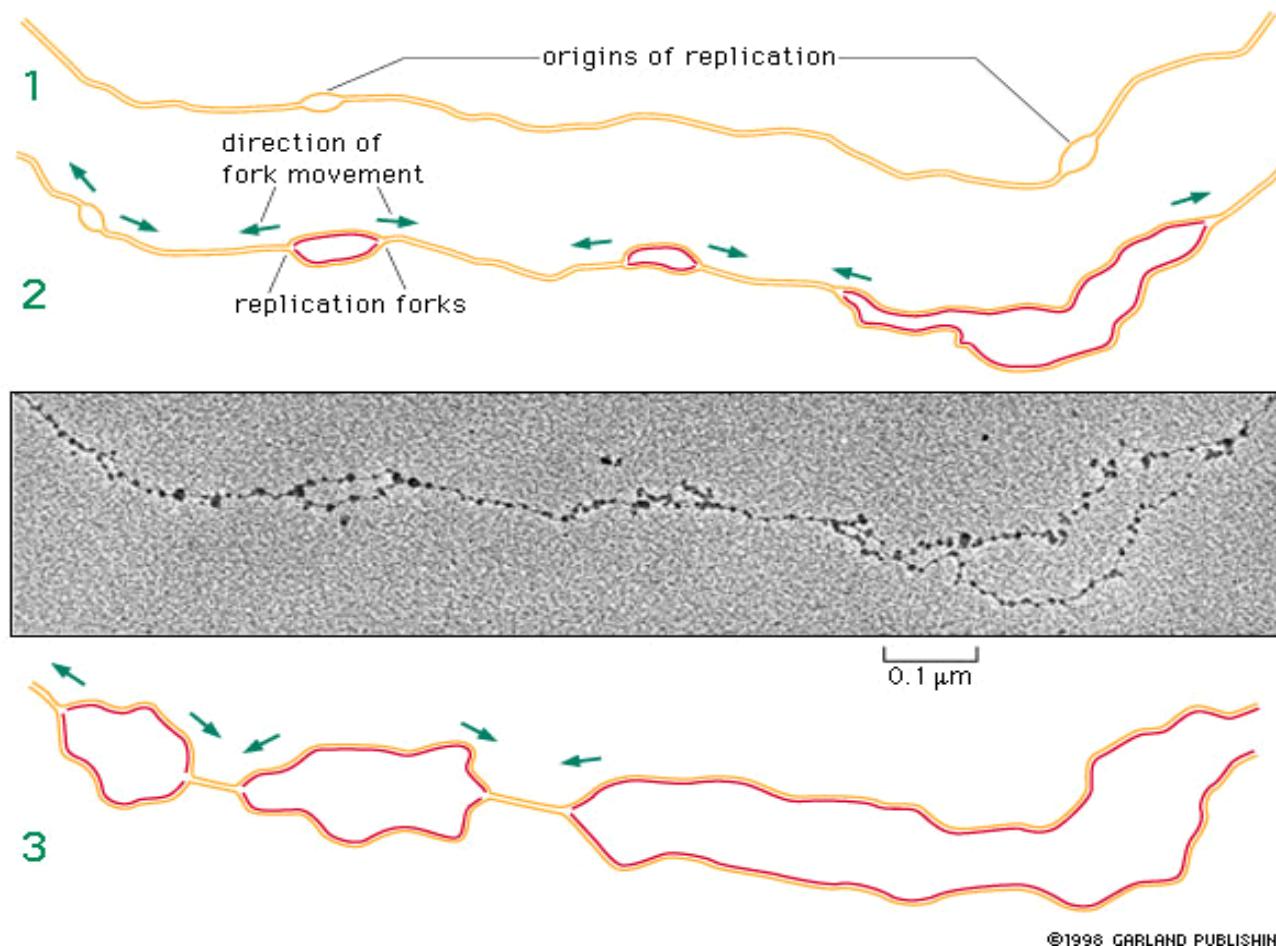
The parental dsDNA needs to be opened to become two ssDNA at the replication origin



# Replication of a circular bacterial DNA occurs from one origin



# In eukaryotes, there are many origins of replication per chromosome



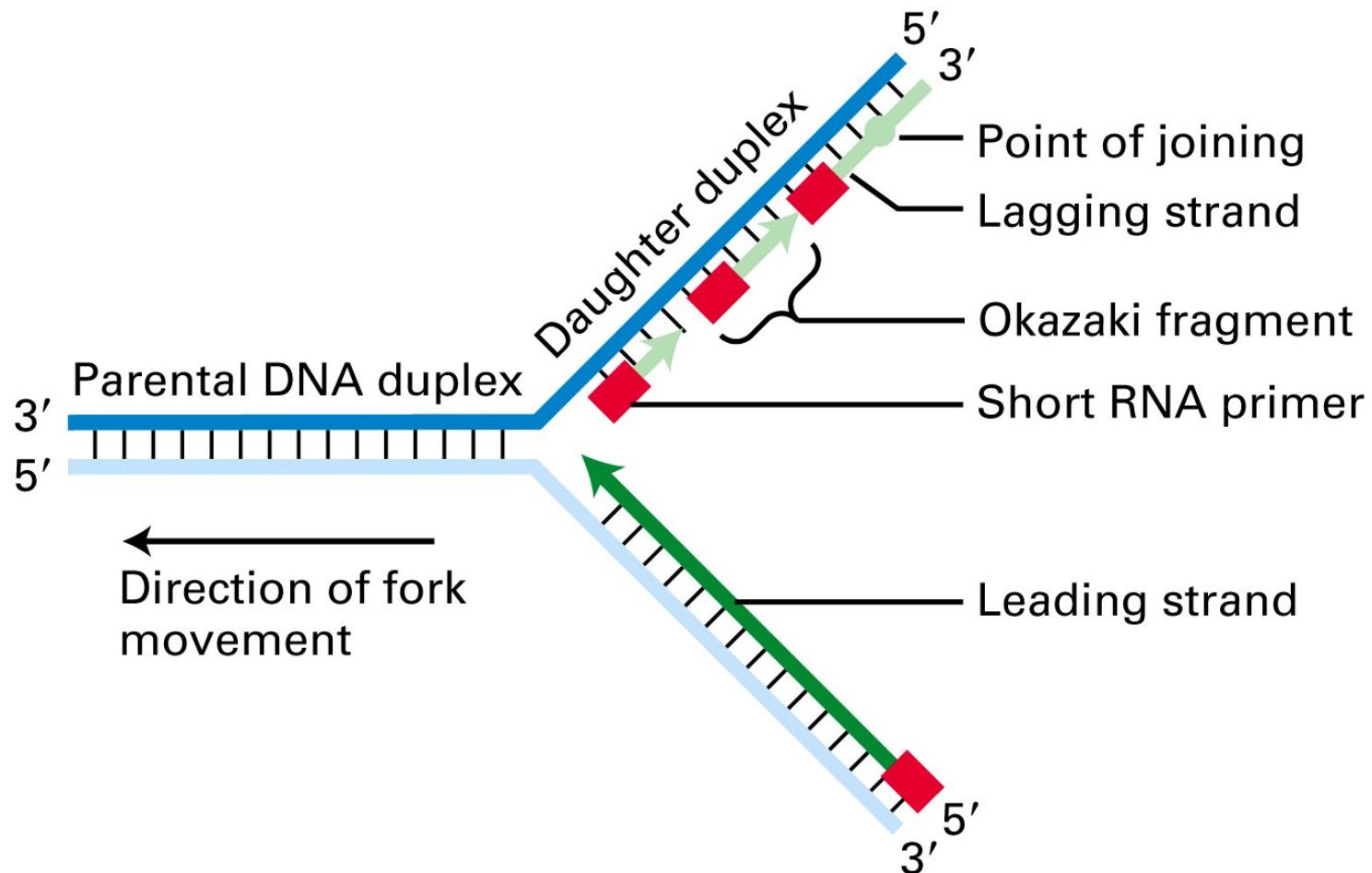
# DNA replication is bidirectional

**Replication fork**—the place where dsDNA “melts” into two ssDNA, there are two forks per origin. Replication proceeds at both ends of the replication fork.

**Leading strand** –the strand that is synthesized continuously

**lagging strand**—the strand that is synthesized discontinuously, resulting in Okazaki fragments

Since DNA synthesis is always 5', one daughter strand is synthesized continuously and the other is synthesized discontinuously.



# DNA synthesis at both replication forks

Leading strand

5'  
3'

lagging strand  
(Okazaki fragments)

3'  
5'

← direction of fork movement →

leading-strand template  
of left-hand fork

5'  
3'

lagging-strand template  
of right-hand fork

3'  
5'

most recently  
synthesized DNA

lagging-strand template  
of left-hand fork

leading-strand template  
of right-hand fork



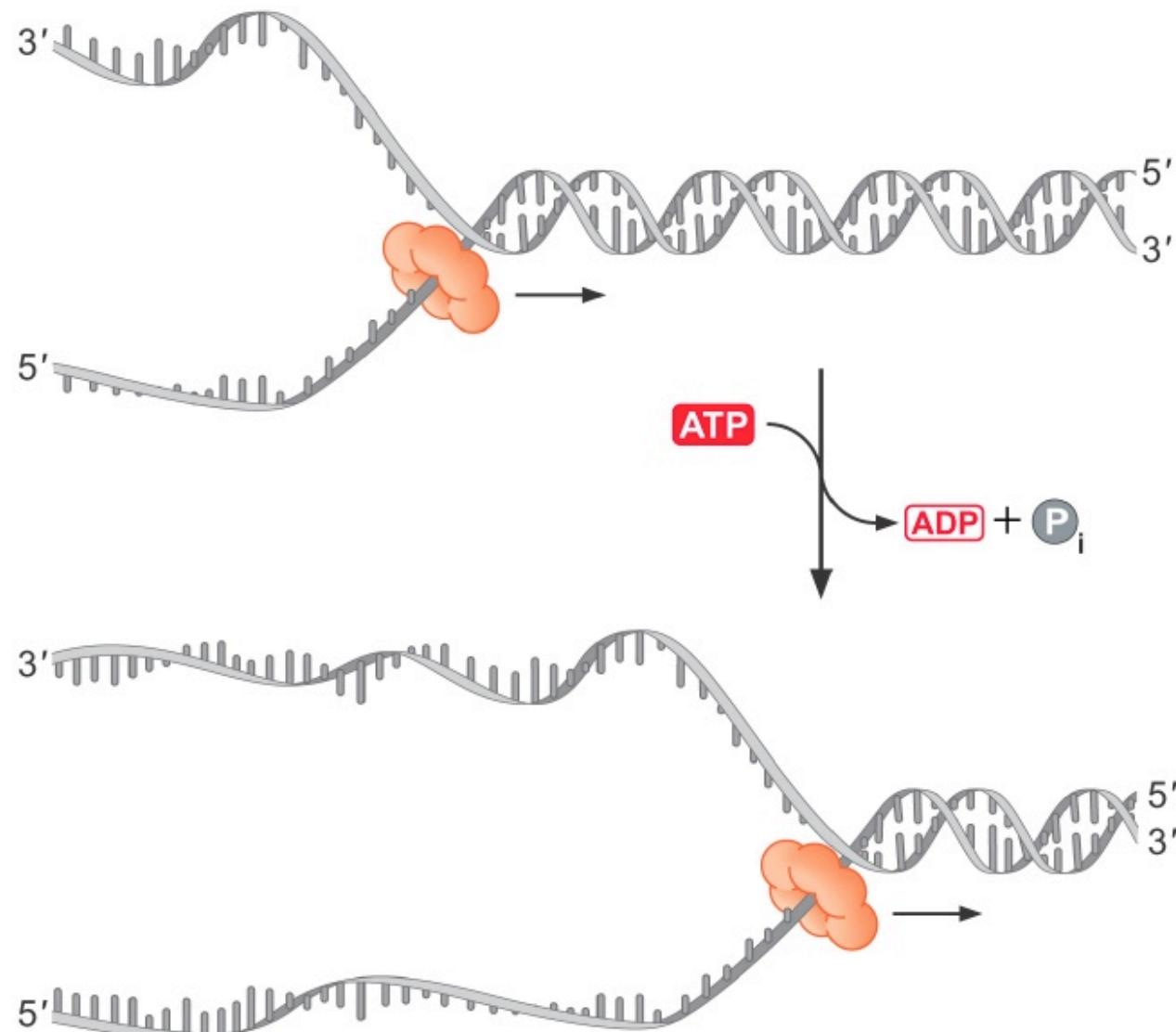
# **Consequences of Replication Fork Structure**

- One DNA strand (the leading strand) grows continuously.
- One strand (the lagging strand) is synthesized in short pieces (called Okazaki fragments) that must be joined together.
- DNA lagging strands must be initiated repeatedly by RNA priming.  
(Primase can initiate an RNA chain.)

# **Key Enzymes and Accessory proteins Involved in DNA replication**

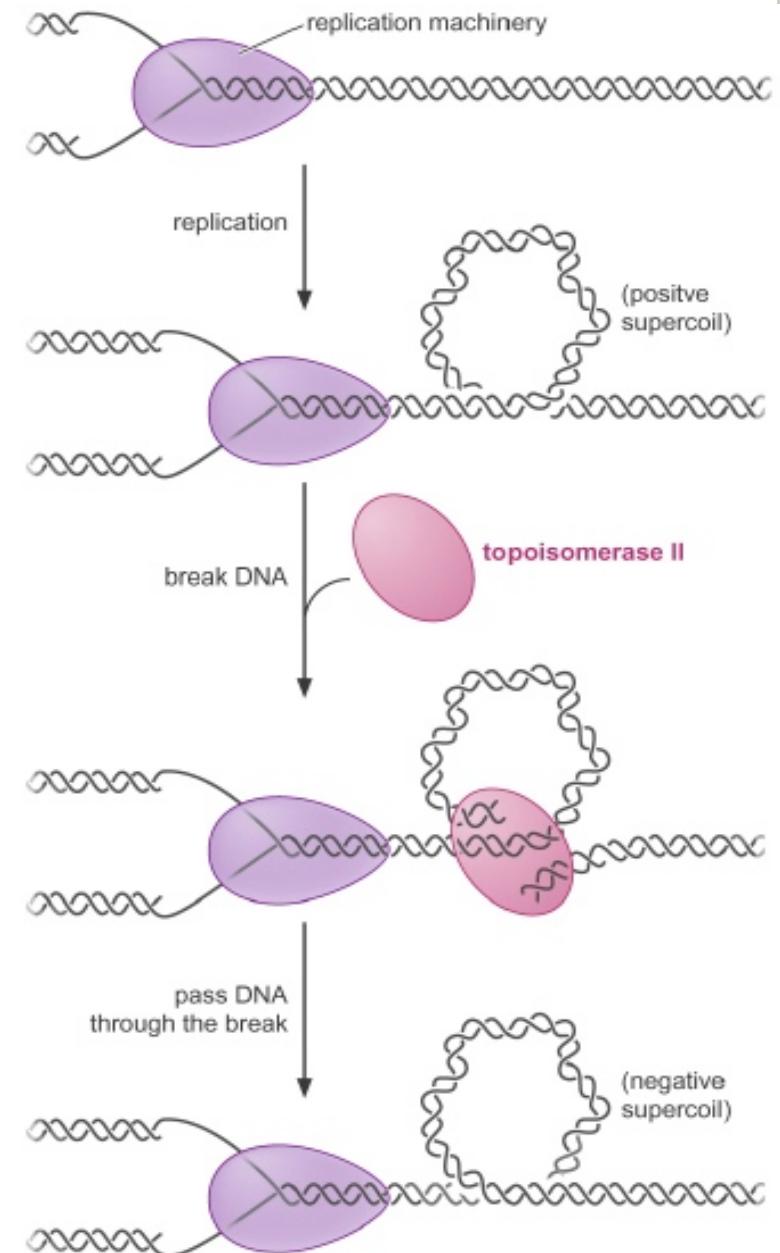
- Helicase
  - DNA topoisomerase
  - Primase
  - DNA polymerase
  - RNase H
  - DNA Ligase
- 
- Single strand DNA binding protein (SSB)
  - Sliding clamp protein

# Helicase unwinds dsDNA to create ssDNA

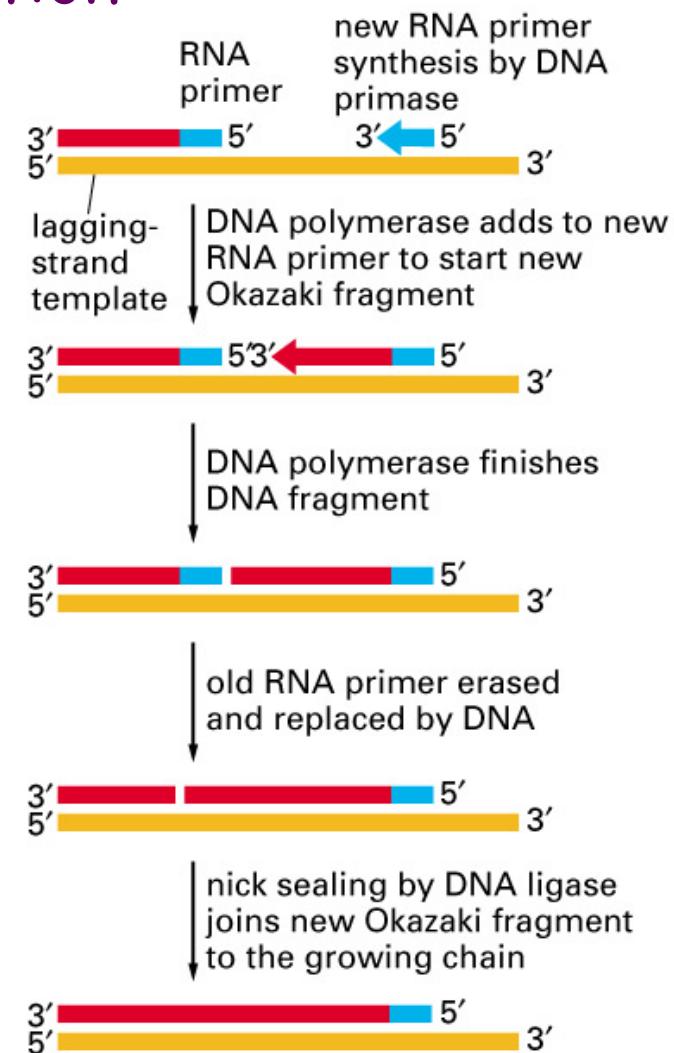
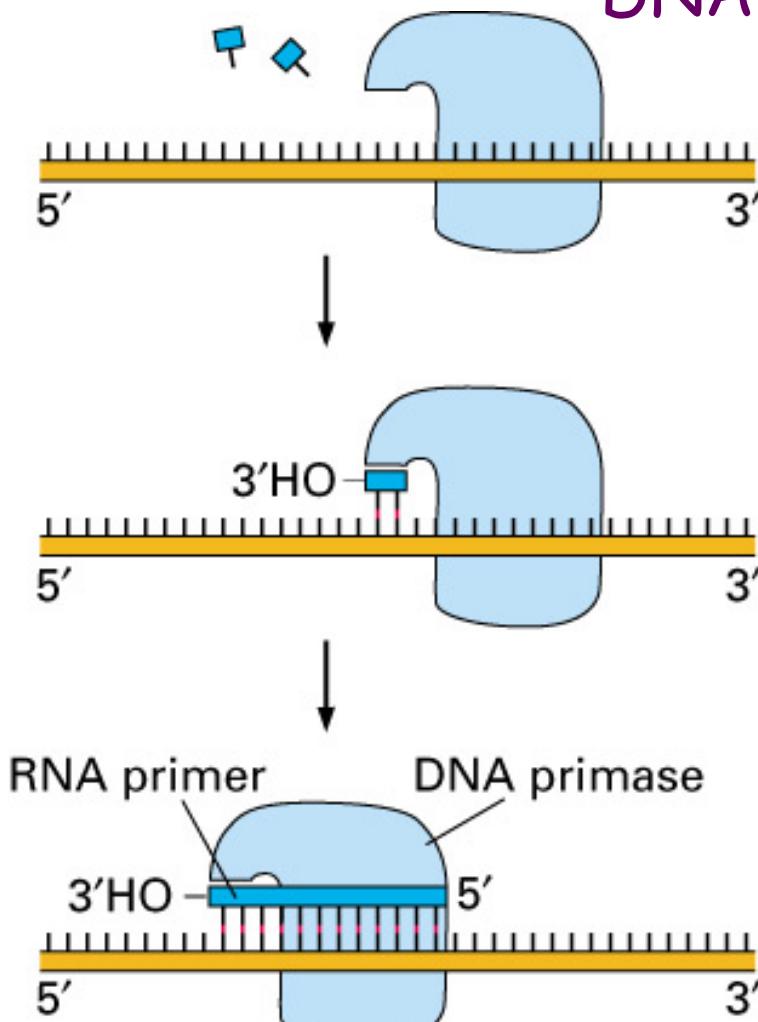


# DNA topoisomerase

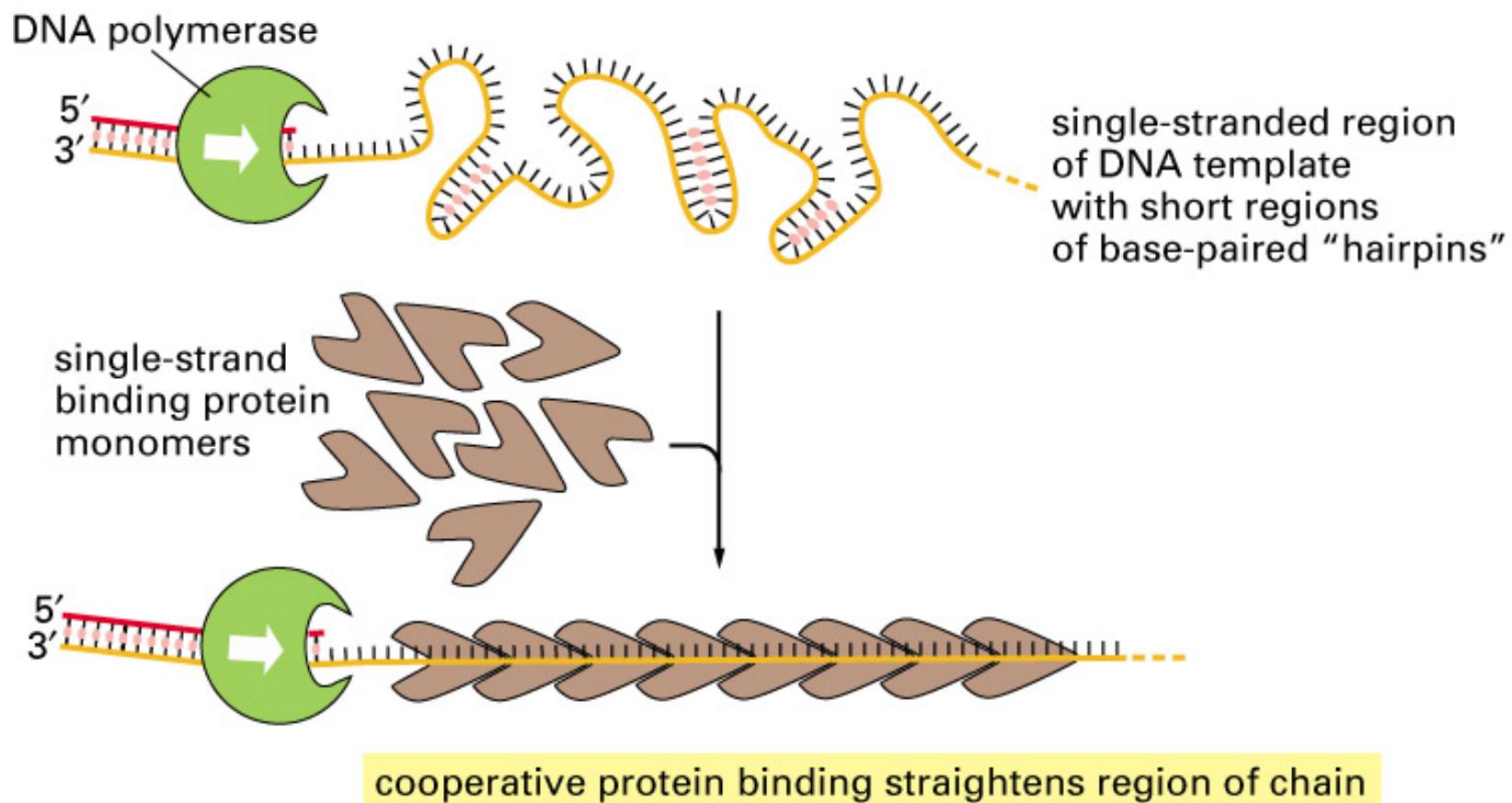
- Unwinding of the double helix at the replication fork causes supercoiling of the DNA helix.
- **DNA topoisomerase** removes supercoils by creating a transient break in one or two strands of DNA, which releases the tension and allow DNA to return to the normal helix (10 bp per turn)



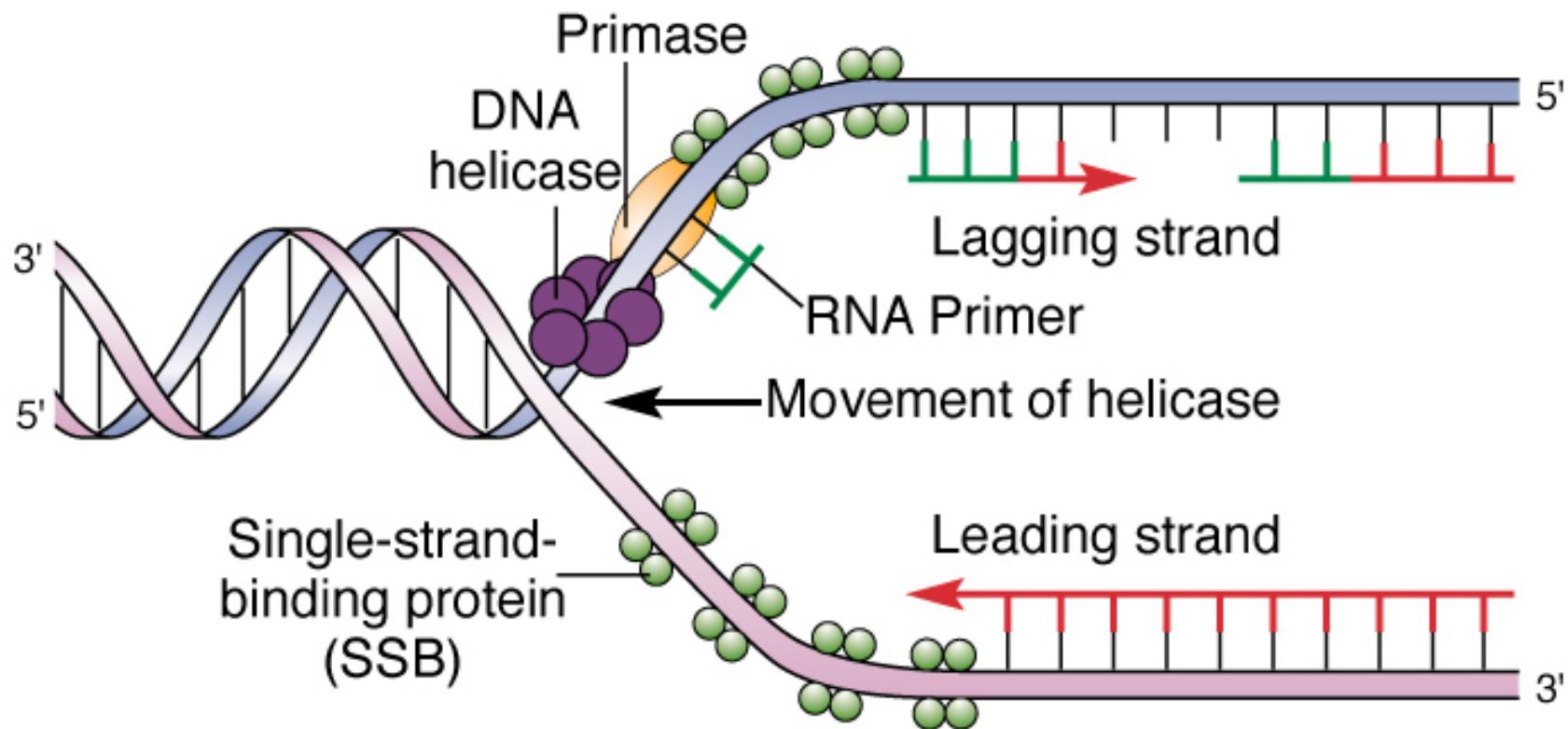
Primase is a RNA polymerase, it synthesizes short RNA primers (5-10 nucleotides long) to help the initiation of DNA replication



# SSB (single strand DNA binding protein) bind and stabilize ssDNA



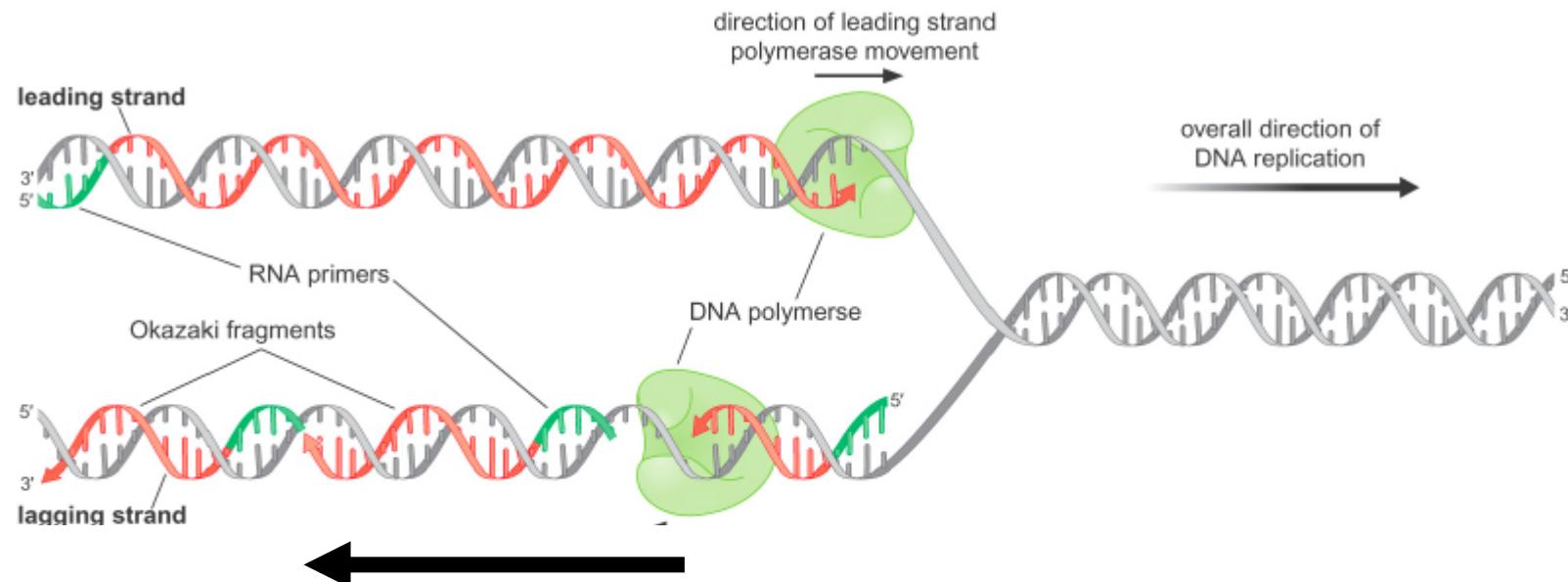
# The role of the DNA helicase, SSB, and primase at the replication fork



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# DNA polymerase

Both prokaryotic and eukaryotic cells have multiple DNA Polymerases. DNA polymerase III is the major E. coli DNA replication enzyme. Polymerases can have three major enzyme activities:



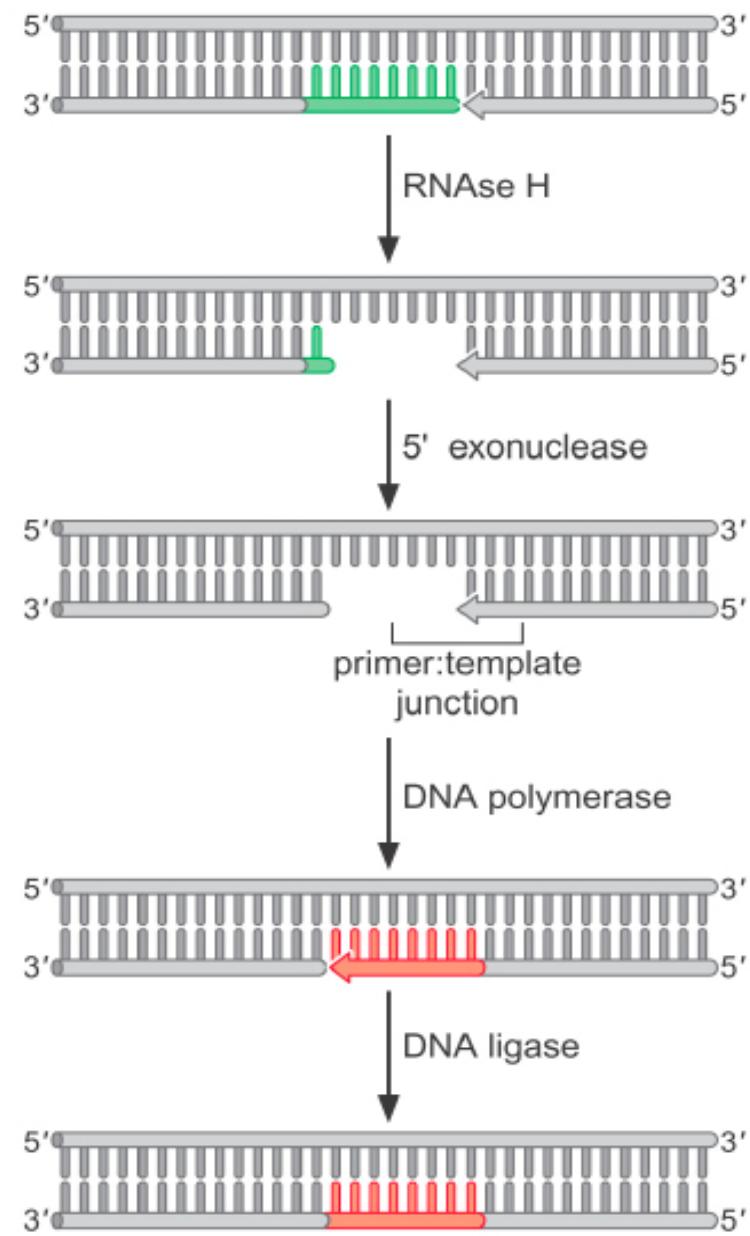
- **5' → 3' polymerase activity – for DNA synthesis  
Mainly Pol III.....**
- **5' → 3' exonuclease activity — for removal of RNA primers  
Mainly Pol I**
- **3' → 5' exonuclease activity – for repairing mistakes**

# RNase H specifically Degrades RNA in the RNA:DNA Hybrid.

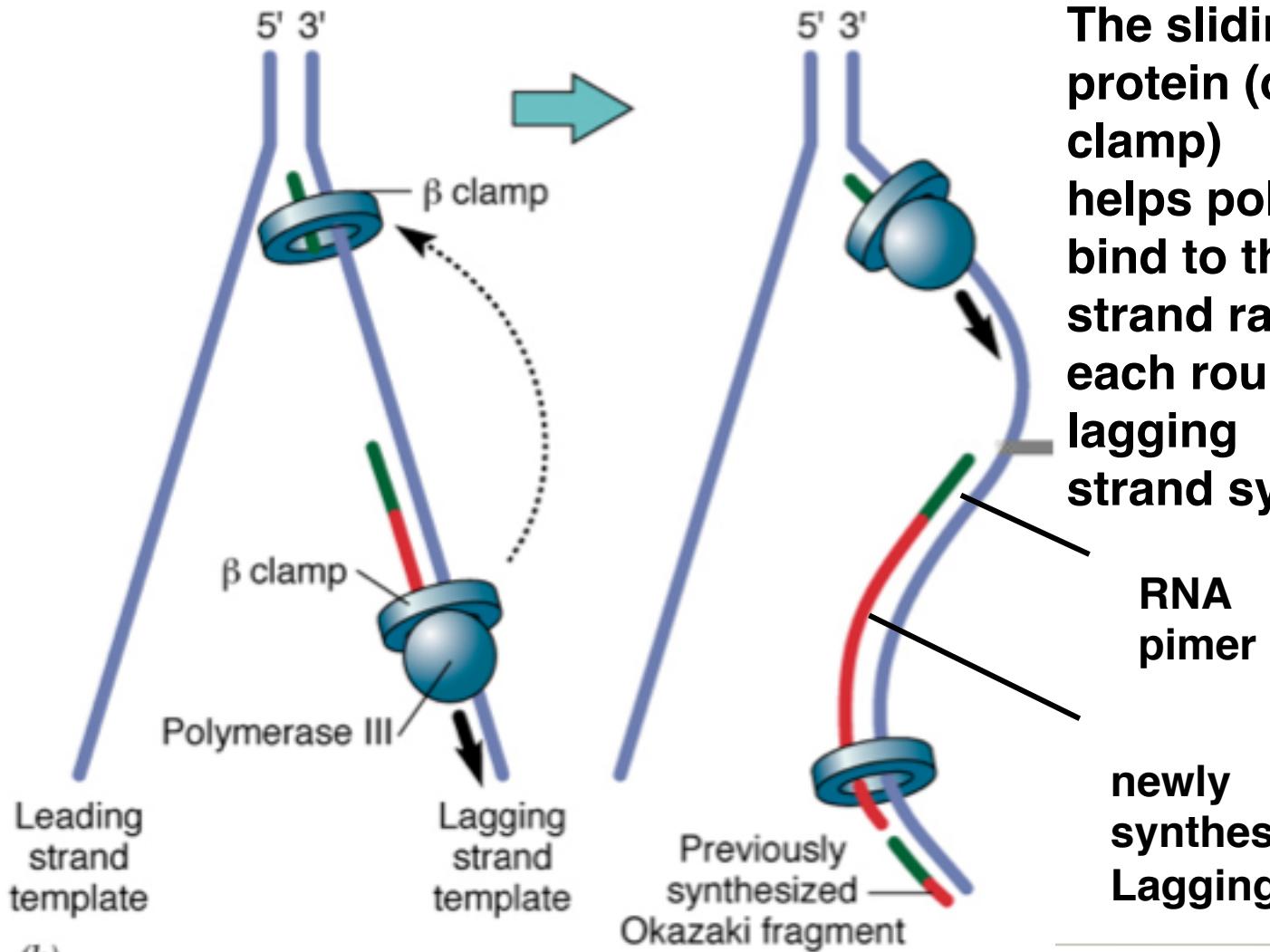
Removal of the RNA primer  
by RNase H and the 5' to 3'  
exonuclease activity of  
DNA polymerase

**DNA ligase** creates a  
phosphodiester bond  
between an adjacent 5'  
phosphate and 3' OH.

Fills the gaps between Okazaki  
fragments to make a  
continuous DNA molecule



# Sliding clamp protein

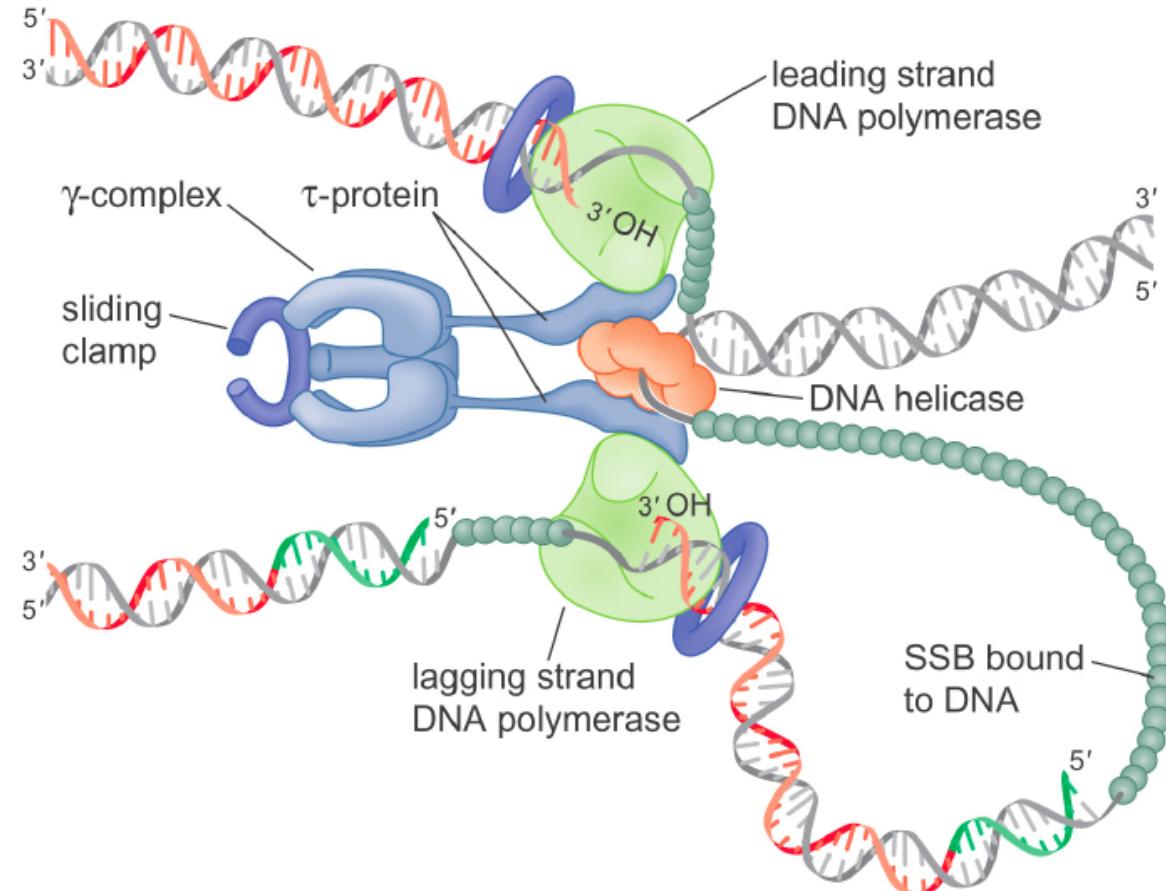


**The sliding clamp protein (or the  $\beta$  clamp) helps polymerase to bind to the template strand rapidly after each round of lagging strand synthesis**

RNA pimer

**newly synthesized Lagging strand**

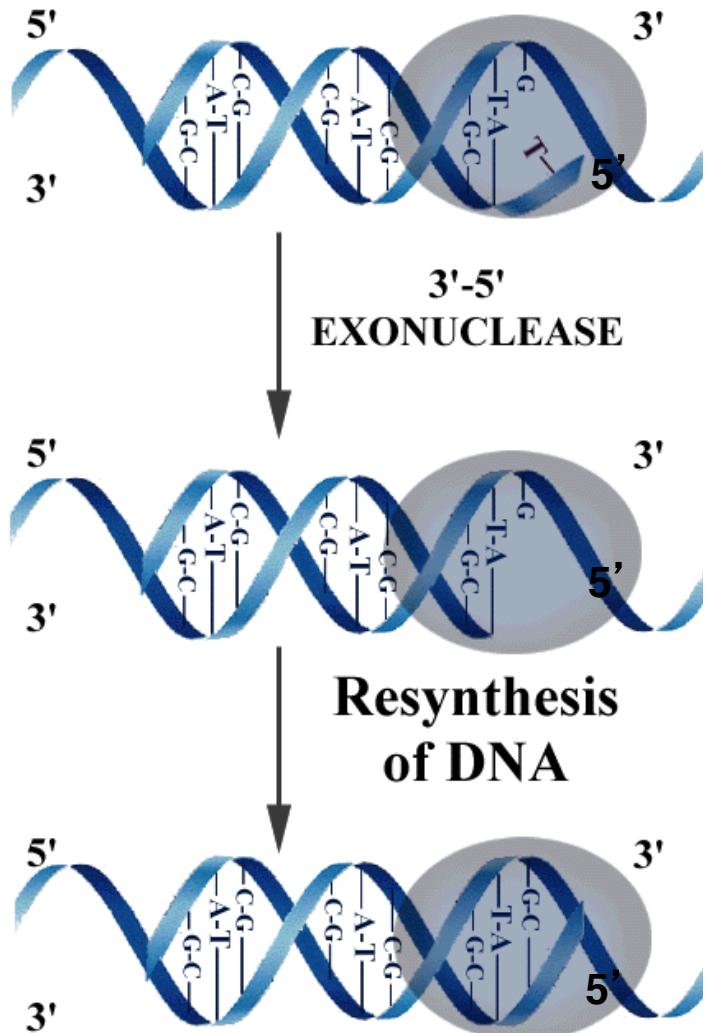
# Schematic model of DNA replication complex



# Summary

- DNA is synthesized in a semi-conservative manner.
- DNA is synthesized bi-directionally
- At each replication fork, DNA is synthesized continuously on the leading strand and discontinuously on the lagging strand.
- RNA is used as a primer.
- RNA primer is removed later and replaced by DNA.

# Proof reading



DNA polymerase has proofreading function ( $3' \rightarrow 5'$  exonuclease activity), which allows it to detect mistakenly incorporated nucleotides, remove them, and replace with the correct nucleotides

-The proofreading is pretty good, but never perfect, that is one of the reason for “spontaneous” mutations in the genome and why organisms have kept evolving.

# If replication is efficient, what causes cancer?

Cancer results from accumulation of multiple mutations in two types of genes and the break-down of regulatory mechanisms of cell growth. The two types of genes involved in carcinogenesis are: **oncogenes** and **tumor suppressor genes**. Mutation in both proto-oncogene and tumor suppressor genes are usually needed to cause cancer, which is also why it is so difficult to treat cancer.

Oncogenes are genes for which **gain-of-expression** or **gain-of-function** cause cancer, they are the altered forms of normal genes called proto-oncogenes. Proto-oncogenes are often **promoters** of cell growth.

Tumor suppressor genes are genes for which the **loss-of-function** causes cancer. Therefore, it is believed that the normal functions of tumor suppressor genes are to suppress tumor formation. Tumor suppressor genes can encode transcription factors, cell-cycle regulators, phosphotases, etc. Tumor suppressor gene products are often **inhibitors** of cell proliferation

# Activation of a proto-oncogene to an oncogene leading to cancer

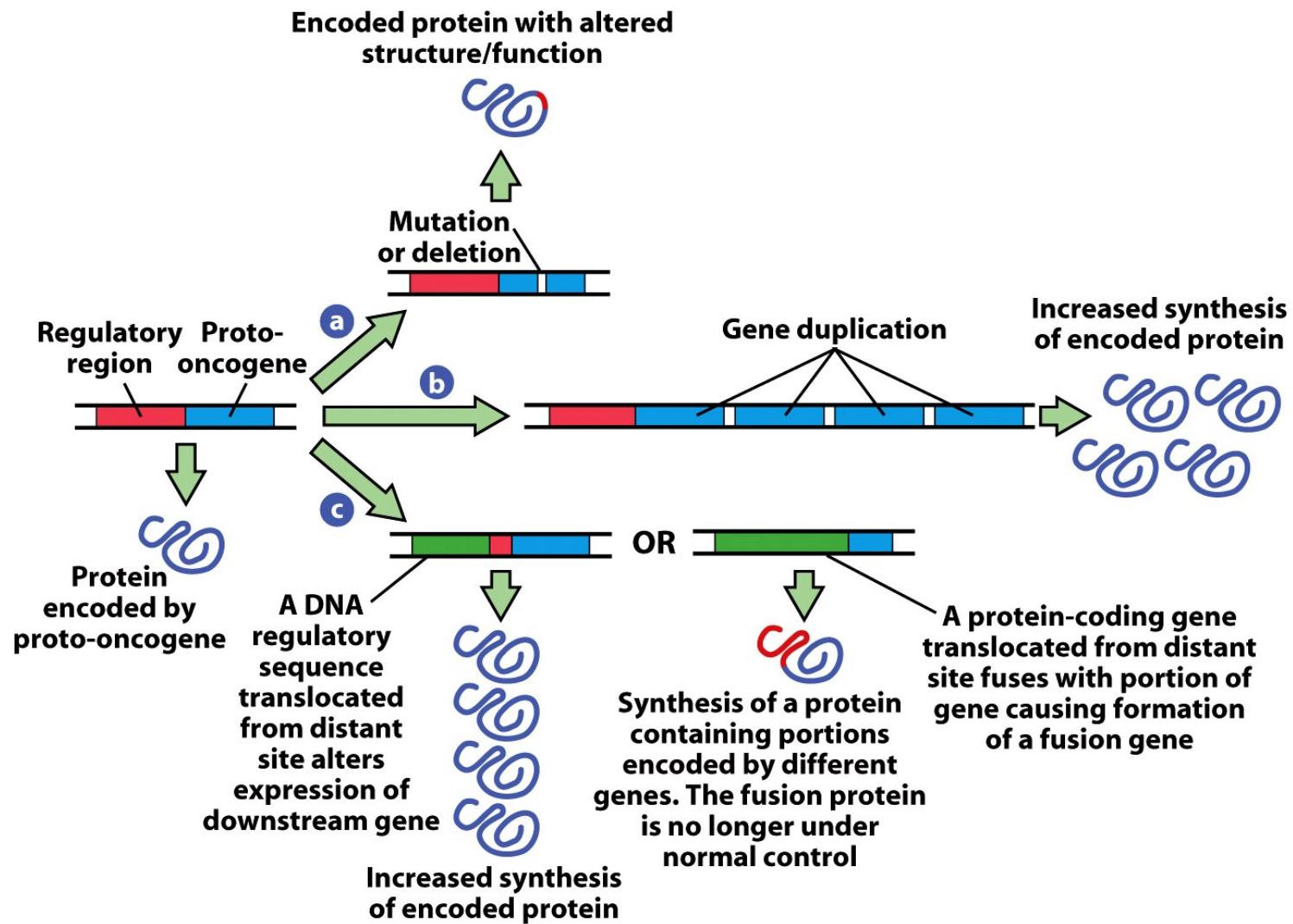


Figure 16-12 Cell and Molecular Biology, 5/e (© 2008 John Wiley & Sons)

# DNA Replication Inhibitors

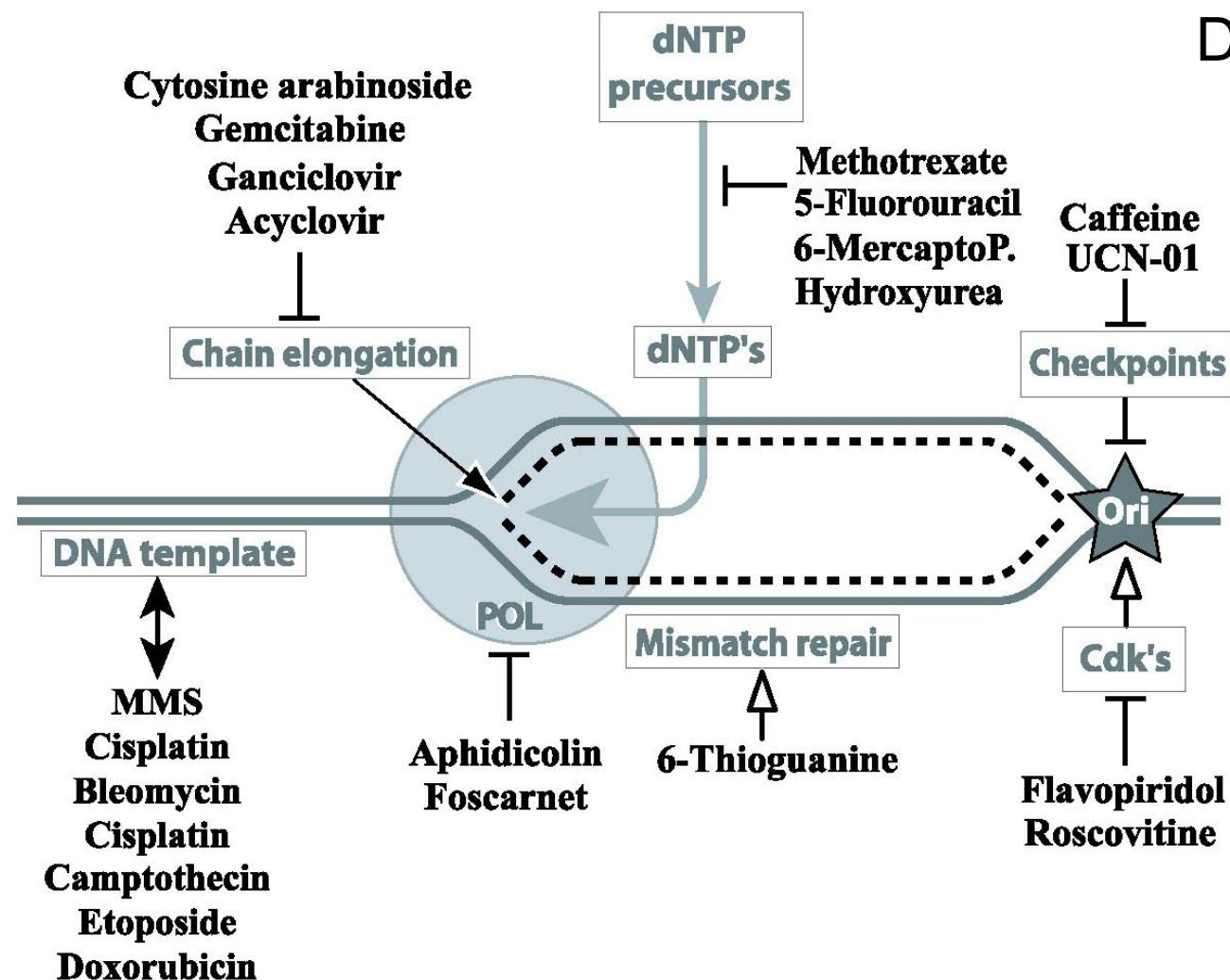


Figure 1. Site of action of commonly used DNA replication inhibitors. 6-Mercaptourine is abbreviated 6-MercaptoP., and 7-hydroxystaurosporine is abbreviated UCN-01.

*DNA Replication and Human Disease* © 2006 Cold Spring Harbor Laboratory Press,  
Chapter 26, Figure 1.

# Implications and Critical Thinking-5

- While leading strand synthesis can proceed until the last nucleotide on the chromosome is copied, the replication fork is incapable of copying the very end of the lagging strand because primase cannot function at the end of a DNA molecule.
- This is a potentially major problem for the cell because it could result in the loss of several bases from the end of one of the chromosomes with every round of DNA replication.
- How does the cell deal with this problem?