LECTURE 17

DNA REPLICATION

Template

dNTPs

Enzymes

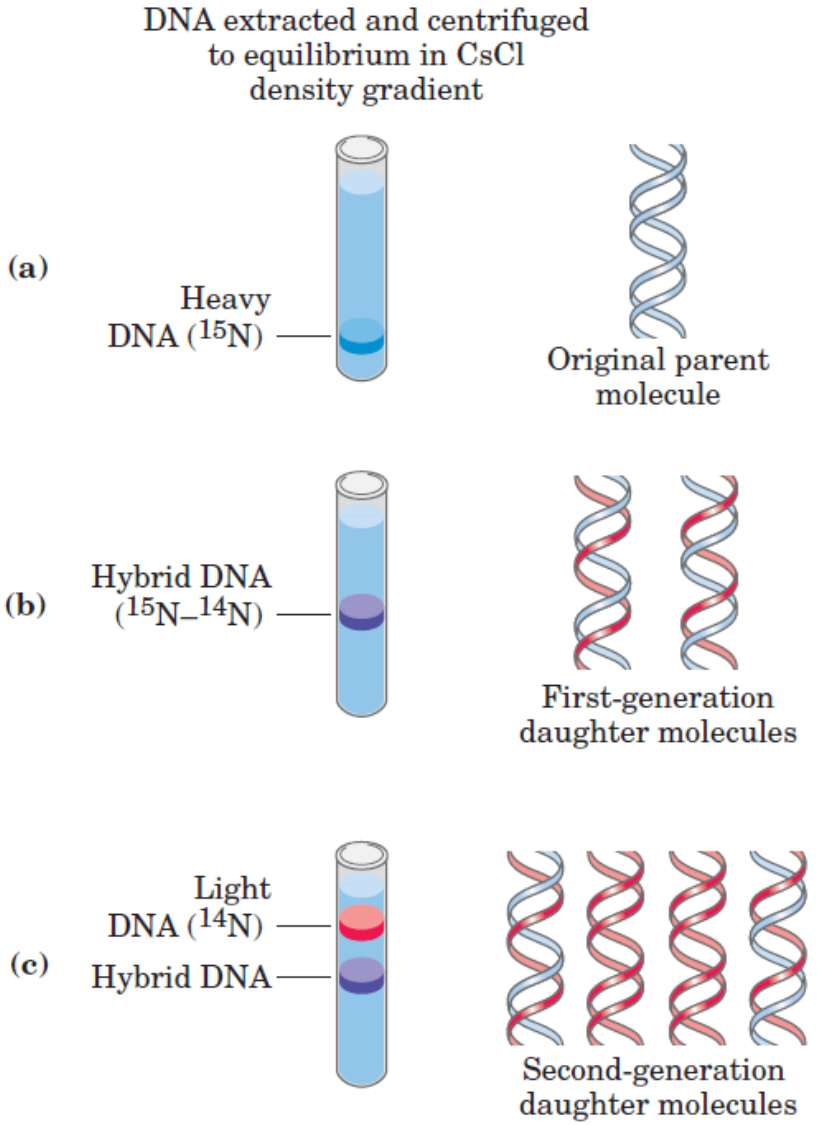
Primer

Fundamental properties of the DNA replication process and the mechanisms used by the enzymes that catalyze it have proved to be essentially identical in all species.

DNA Replication Is Semiconservative

Each DNA strand serves as a template for the synthesis of a new strand, producing two new DNA molecules, each with one new strand and one old strand.

This is **semiconservative replication**.

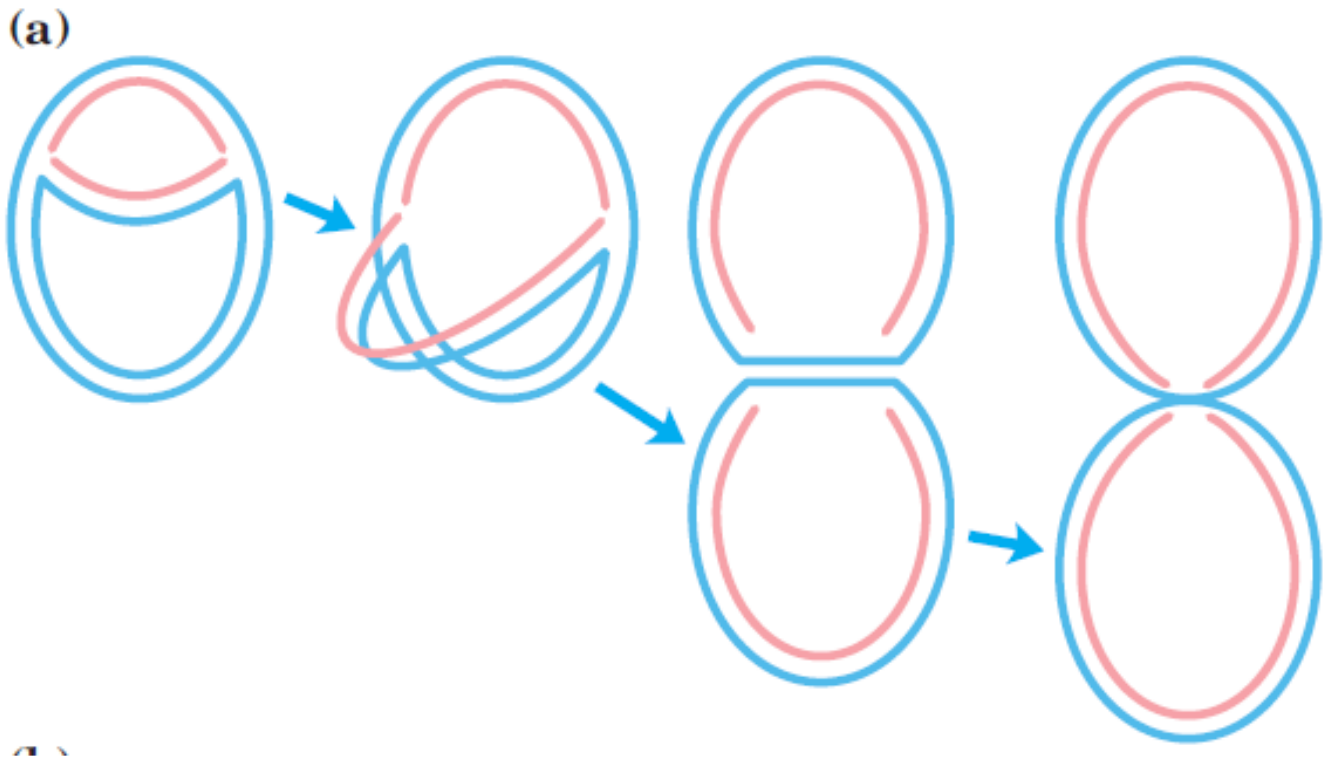


- Are the parent DNA strands completely unwound before each is replicated?
- Does replication begin at random places or at a unique point?
- After initiation at any point in the DNA, does replication proceed in one direction or both?

Replication Begins at an Origin and Usually Proceeds Bidirectionally

One or both ends of the loop are dynamic points, termed **replication forks**, where parent DNA is being unwound and the separated strands quickly replicated.

The replication loops always initiate at a unique point, which is termed an **origin**.

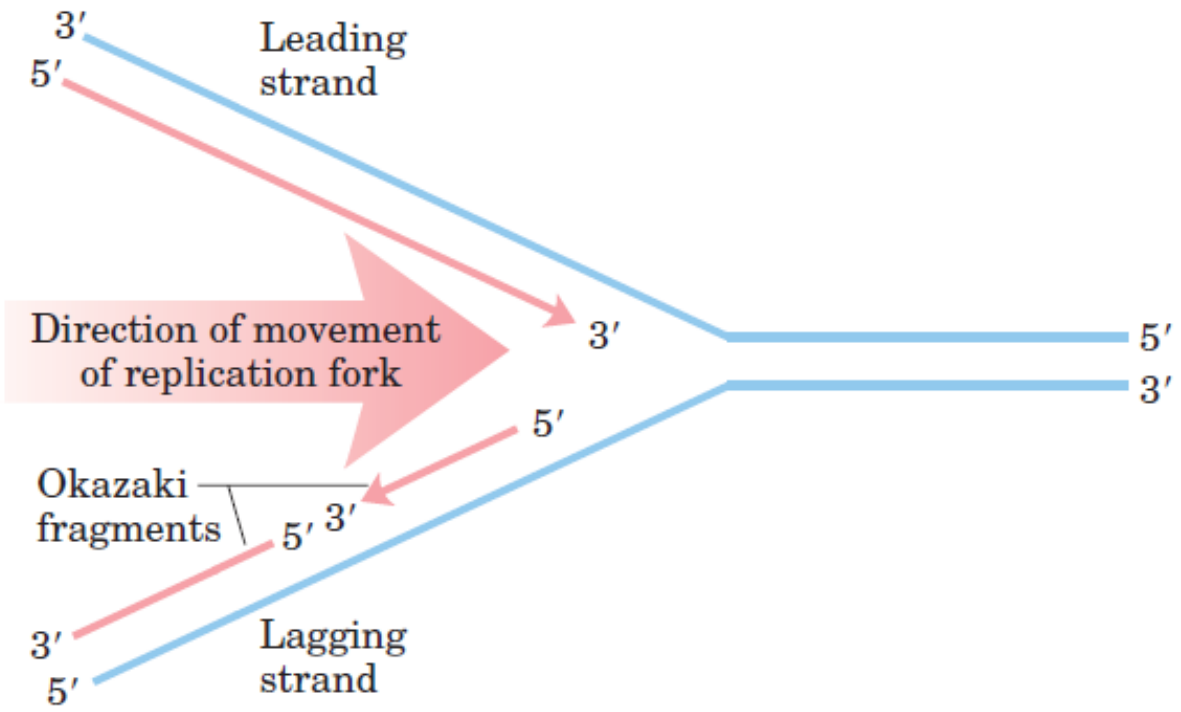


DNA Synthesis Proceeds in a 5’-3’ Direction and Is Semi discontinuous

The continuous strand, or **leading strand**, is the one in which 5’-3’ synthesis proceeds in the *same* direction as replication fork movement.

The discontinuous strand, or **lagging strand**, is the one in which 5’-3’ synthesis proceeds in the direction *opposite* to the direction of fork movement.

Okazaki fragments range in length from a few hundred to a few thousand nucleotides, depending on the cell type



Several Enzymes are required for DNA replication

- Nucleases
- DNAases –Exonucleases/Endonucleases

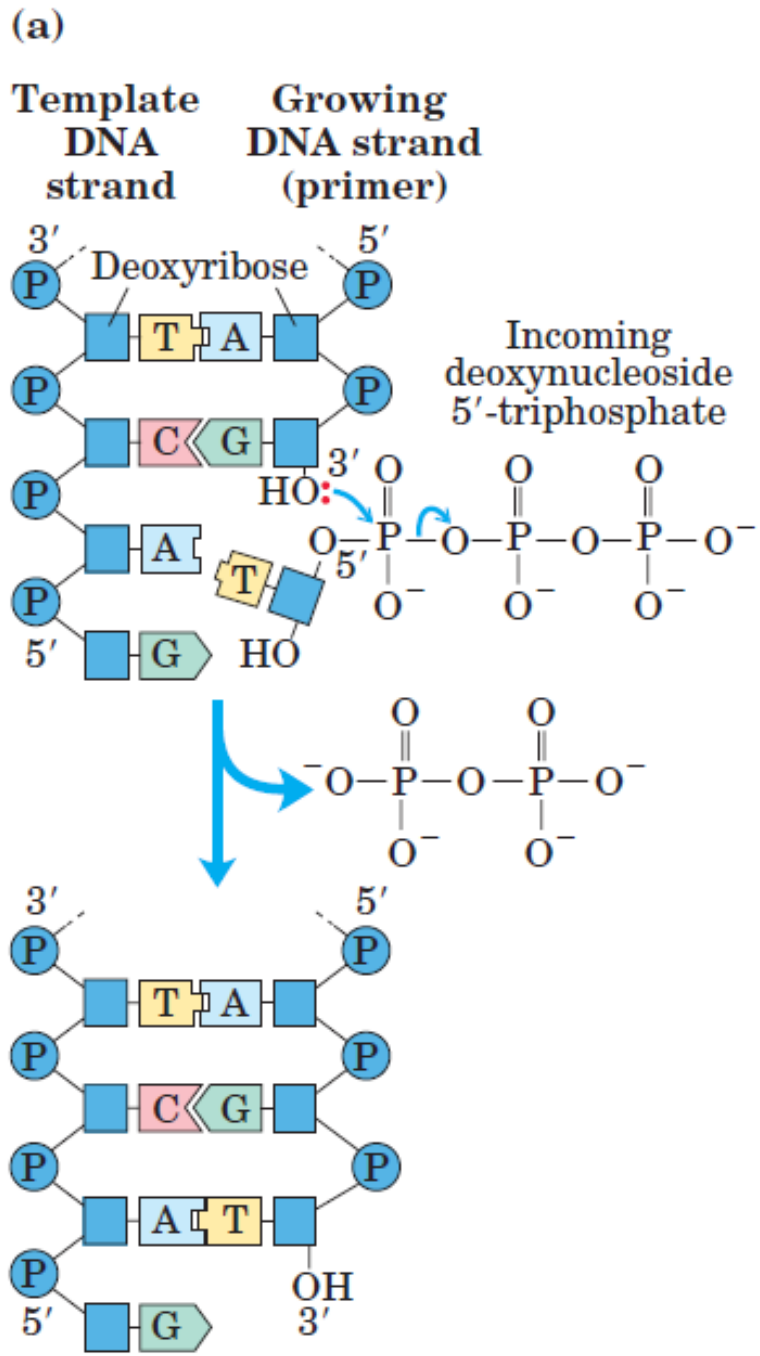
- DNA polymerase

Requires a template and a primer

A primer is a strand segment (complementary to the template) with a free 3-hydroxyl group to which a nucleotide can be added

After adding a nucleotide to a growing DNA strand, a DNA polymerase either dissociates or moves along the template and adds another nucleotide

The average number of nucleotides added before a polymerase dissociates defines its **processivity**.


(a)
Template DNA strand
Growing DNA strand (primer)
3′
5′
Deoxyribose
Incoming deoxynucleoside 5′-triphosphate
HO
OH
P
T
A
C
G
A
T
G
O
O⁻

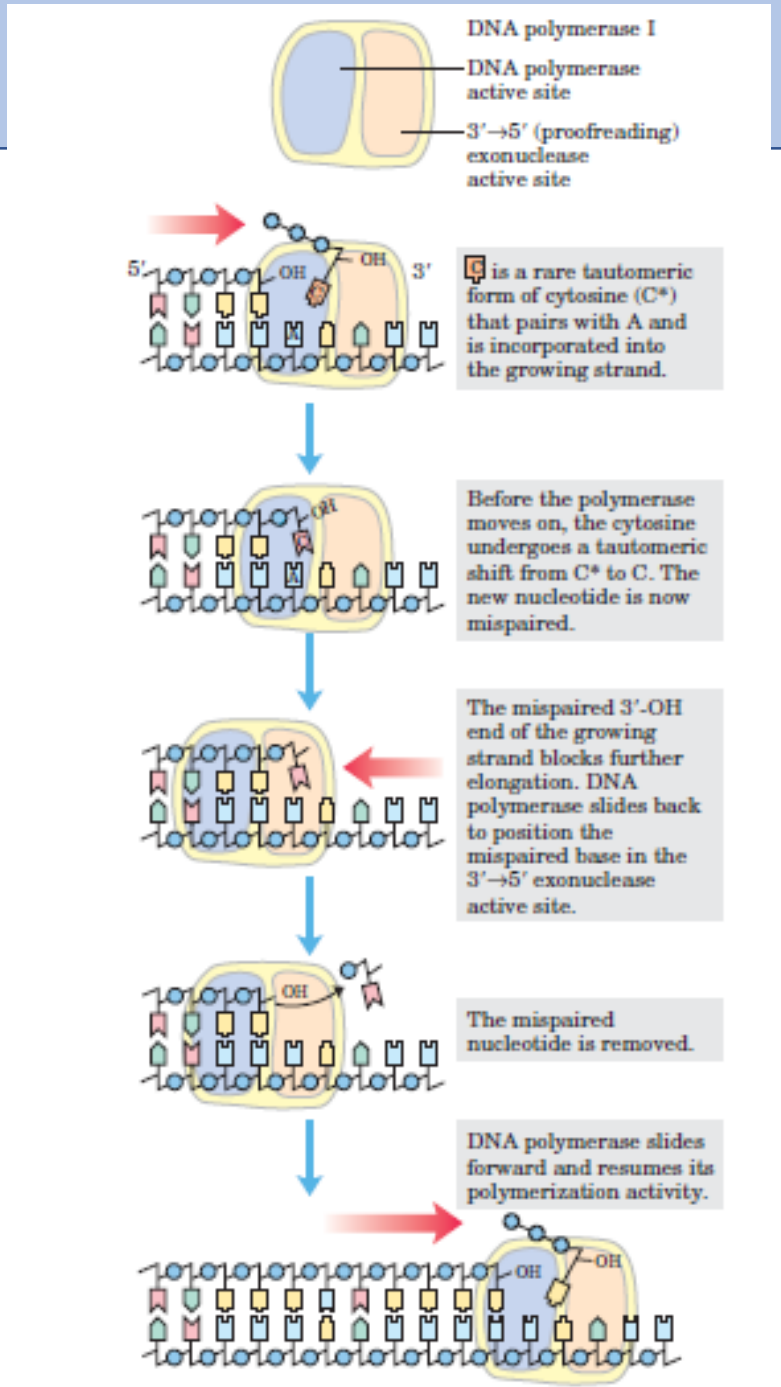
Replication Is Very Accurate

Replication proceeds with an extraordinary degree of **fidelity.**

In *E. coli*, a mistake is made only once for every 10^9 to 10^{10} nucleotides added.

For the *E. coli* chromosome of 4.6 X10^6 bp, this means that an error occurs only once per 1,000 to 10,000 replications.

One mechanism intrinsic to virtually all DNA polymerases is a separate 3’-5’ exonuclease activity that double-checks each nucleotide after it is added.


DNA polymerase I
DNA polymerase active site
3′→5′ (proofreading) exonuclease active site
5′
OH
OH
3′
C* is a rare tautomeric form of cytosine (C*) that pairs with A and is incorporated into the growing strand.
Before the polymerase moves on, the cytosine undergoes a tautomeric shift from C* to C. The new nucleotide is now mispaired.
The mispaired 3′-OH end of the growing strand blocks further elongation. DNA polymerase slides back to position the mispaired base in the 3′→5′ exonuclease active site.
OH
The mispaired nucleotide is removed.
DNA polymerase slides forward and resumes its polymerization activity.
OH
OH

E. coli Has at Least Five DNA Polymerases

TABLE 25–1 Comparison of Three DNA Polymerases of *E. coli*

	DNA polymerase		
	I	**II**	**III**
Structural gene*	*polA*	*polB*	*polC (dnaE)*
Subunits (number of different types)	1	7	≥10
M_r	103,000	88,000†	791,500
3′→5′ Exonuclease (proofreading)	Yes	Yes	Yes
5′→3′ Exonuclease	Yes	No	No
Polymerization rate (nucleotides/s)	16–20	40	250–1,000
Processivity (nucleotides added before polymerase dissociates)	3–200	1,500	≥500,000

DNA Replication Requires Many Enzymes and Protein Factors

Replication in *E. coli* requires not just a single DNA polymerase but 20 or more different enzymes and proteins, each performing a specific task

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The entire complex has been termed the **DNA replicase system** or **replisome**.

Access to the DNA strands that are to act as templates requires separation of the two parent strands.

This is generally accomplished by **helicases**, enzymes that move along the DNA and separate the strands, using chemical energy from ATP.

Strand separation creates topological stress in the helical DNA structure which is relieved by the action of **topoisomerases**.

The separated strands are stabilized by **DNA binding proteins**.

Before DNA polymerases can begin synthesizing DNA, primers must be present on the template—generally short segments of RNA synthesized by enzymes known as **primases**.

Ultimately, the RNA primers are removed and replaced by DNA; in *E. coli*, this is one of the many functions of **DNA polymerase I.**

After an RNA primer is removed and the gap is filled in with DNA, a nick remains in the DNA backbone in the form of a broken phosphodiester bond.

These nicks are sealed by **DNA ligases**.