Genetic Information: DNA replication

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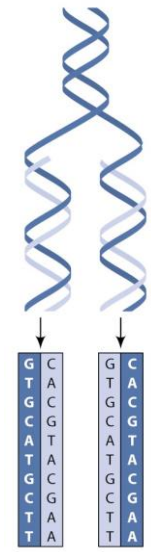
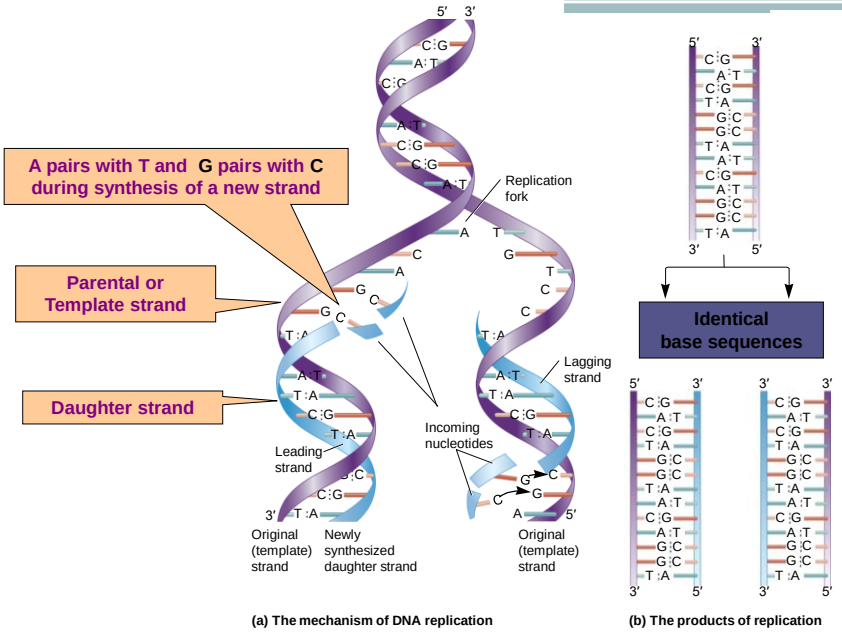
DNA Replication

- Replication of DNA is vital to the transmission of genomes and the genes they contain from one cell generation to the other.
- It must be executed precisely if we want genetic continuity cells.
- It is a huge task because there is so much to replicate.
- Even an error rate of 10^{-6} will lead to 3000 errors during replication.
- It cannot be error free but we still need a very reliable system.

Semiconservative DNA replication was proposed by Watson and Crick

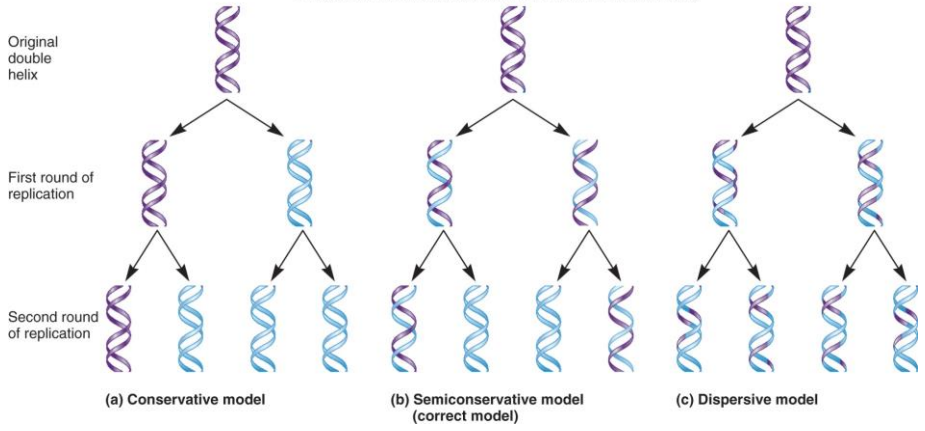
- Watson and Crick thought that because of the arrangement and the chemical properties of the DNA , each strand of the double helix could serve as a template for the synthesis of its complement.
- If the helix is unwound, each nucleotide along the parent strand would have an affinity for its complementary nucleotide. The affinity and the complementarity would be due to the hydrogen bonds.
- The nucleotides would then be linked together into polynucleotide chains along their templates.
- Each replicated DNA molecule would consist of one "old" strand and one "new" strand, hence the name **semiconservative replication**.



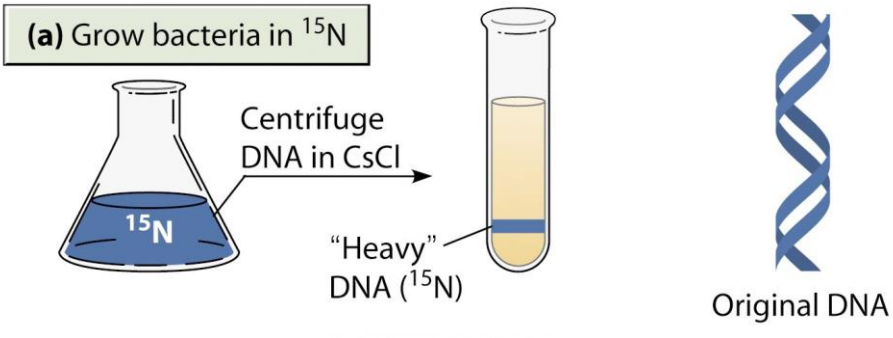


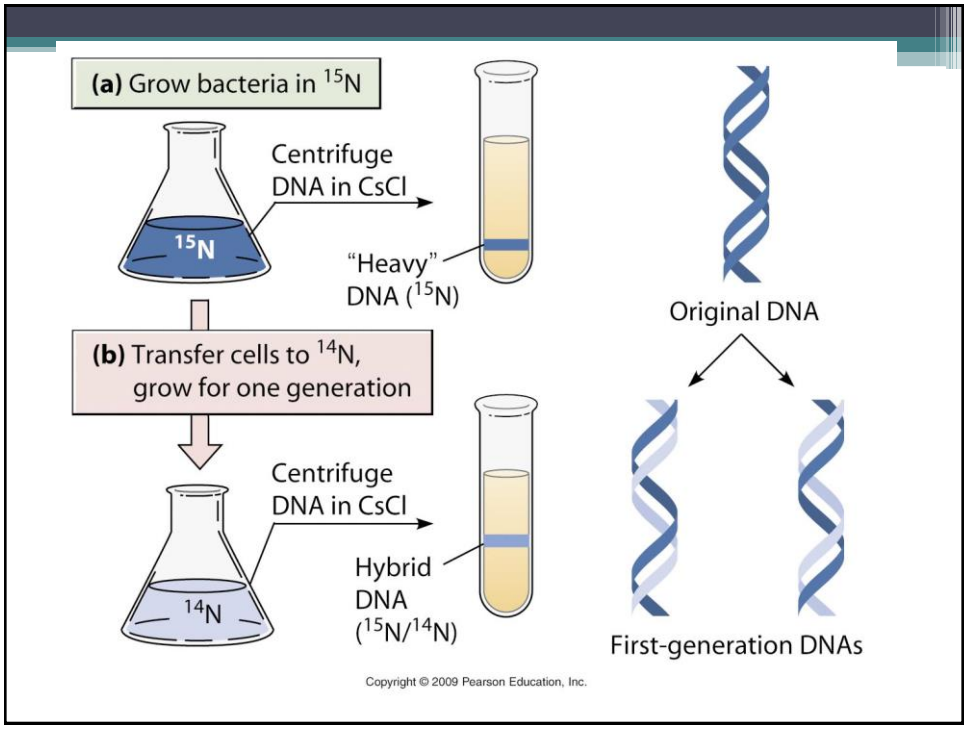
(a) The mechanism of DNA replication

(b) The products of replication



Meselson-Stahl experiment providing evidence for semiconservative DNA replication




(a) Grow bacteria in ^{15}N
Centrifuge DNA in CsCl
^{15}N
"Heavy" DNA (^{15}N)
Original DNA
(b) Transfer cells to ^{14}N, grow for one generation
Centrifuge DNA in CsCl
^{14}N
Hybrid DNA ($^{15}N/^{14}N$)
First-generation DNAs
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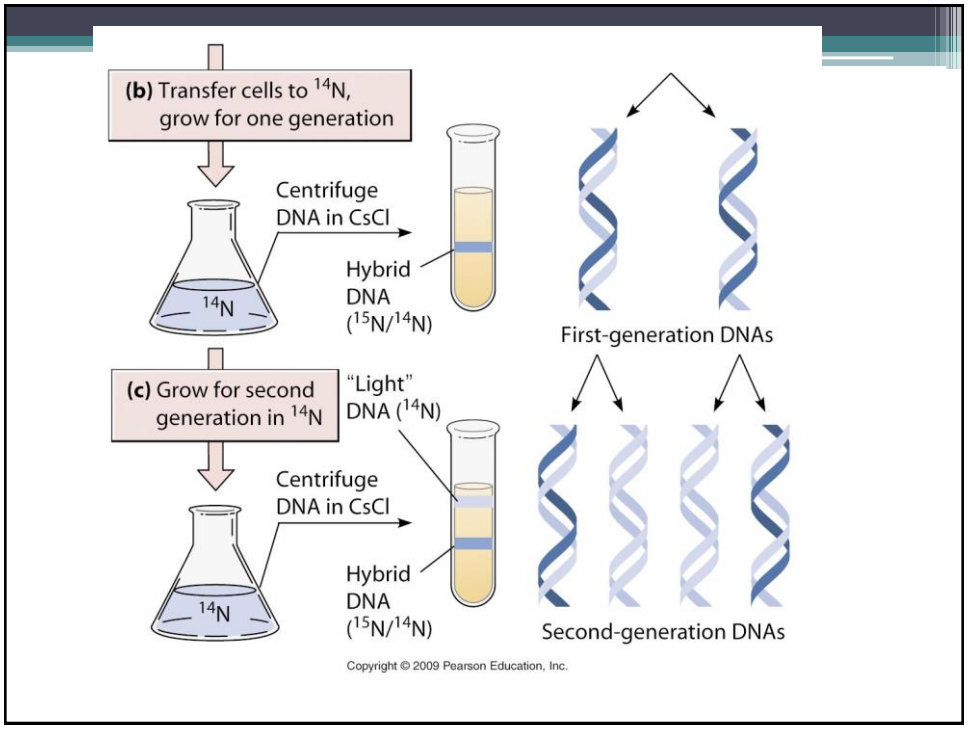
(b) Transfer cells to ^{14}N, grow for one generation
Centrifuge DNA in CsCl
^{14}N
Hybrid DNA ($^{15}N/^{14}N$)
First-generation DNAs
(c) Grow for second generation in ^{14}N
"Light" DNA (^{14}N)
Centrifuge DNA in CsCl
^{14}N
Hybrid DNA ($^{15}N/^{14}N$)
Second-generation DNAs
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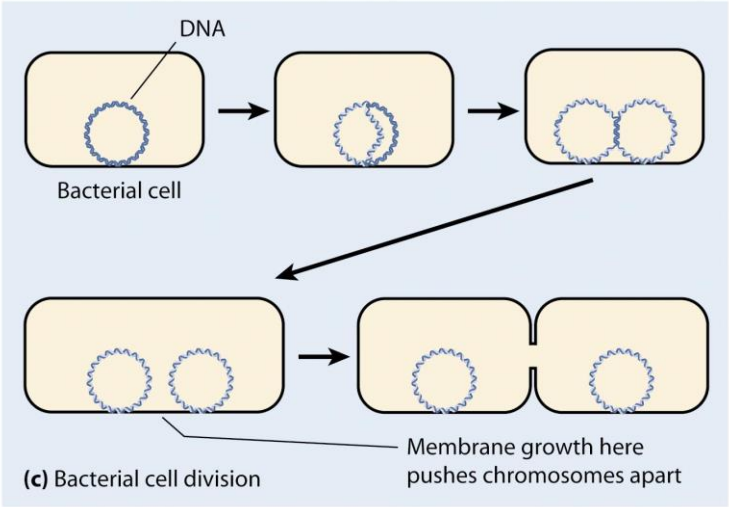
DNA replication characterization

- In 1955, Arthur Kornberg and his colleges characterized DNA replication in bacteria because bacteria replication machinery was though to be less complex than eukaryotes.
- Bacteria contains three different polymerases, DNA polymerase (pol) I, II, and III.
- Replication of the *E. coli* genome is the job of pol III. DNA pol I is the first one that was identified.
- DNA polymerase requires a number of additional accessory proteins.

Questions that can come to your mind when thinking about DNA replication.

- Where along the chromosome does the replication begin? Is the location random or specific?
- Is there only one origin of replication per chromosome?
- After the start of the replication, is the replication unidirectional or bidirectional?
- In eukaryotes, DNA replication is semiconservative, bidirectional with multiple origins of replication in on each chromosome.

DNA replication in bacteria



The first experiments

- Kornberg and his colleagues isolated an enzyme that was able to direct DNA synthesis in vitro. They called this enzyme DNA polymerase I (Kornberg enzyme).
- They found 5 components were needed for DNA to be synthesized:
  - All four dNTPs (if any of them were missing or if the derivatives were used no measureable synthesis occurred).
  - DNA pol I (the enzyme).
  - DNA (acts as a template).
  - A primer (they used Digested DNA but the primer is usually an RNA).
  - Magnesium ions (needed for optimal DNA pol activity).

DNA replication

Main points to consider

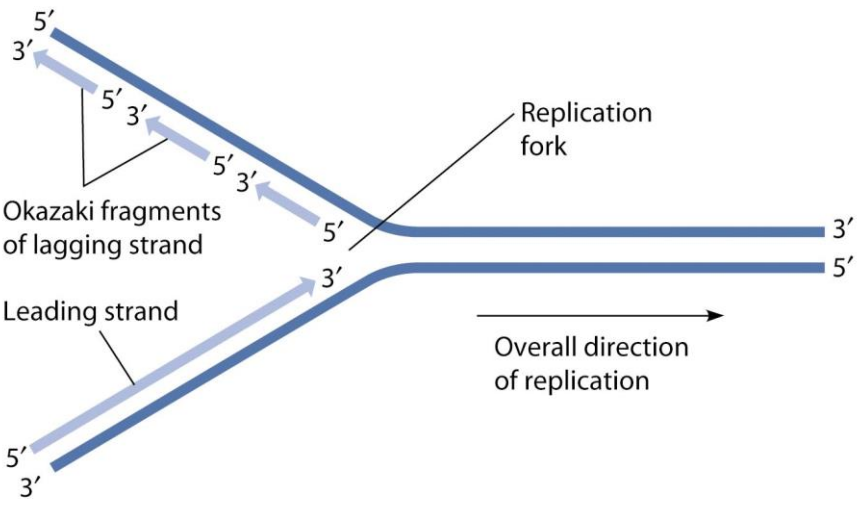
- Semiconservative
- Bidirectional
- Direction of synthesis is 5' to 3'
- Two strands, leading strand and lagging strand
- Primers
- Origin of replication

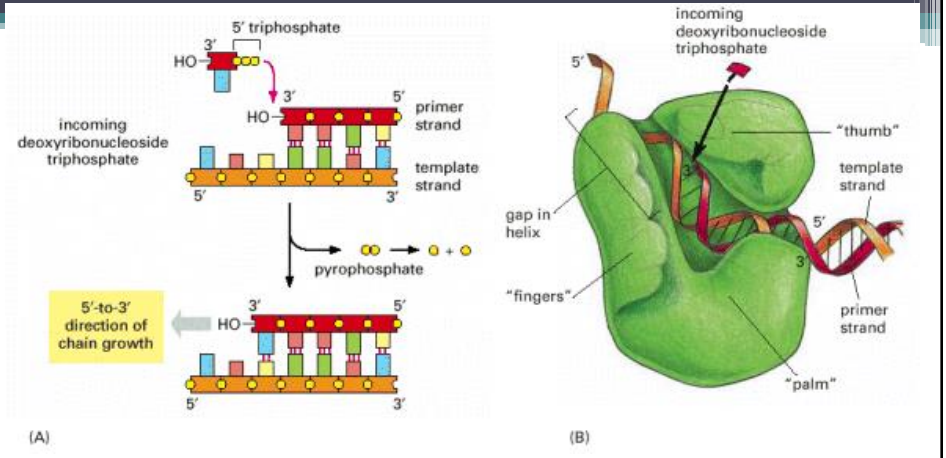
Three stages of DNA replication

- Initiation
- Elongation
- Termination

Things to do during replication

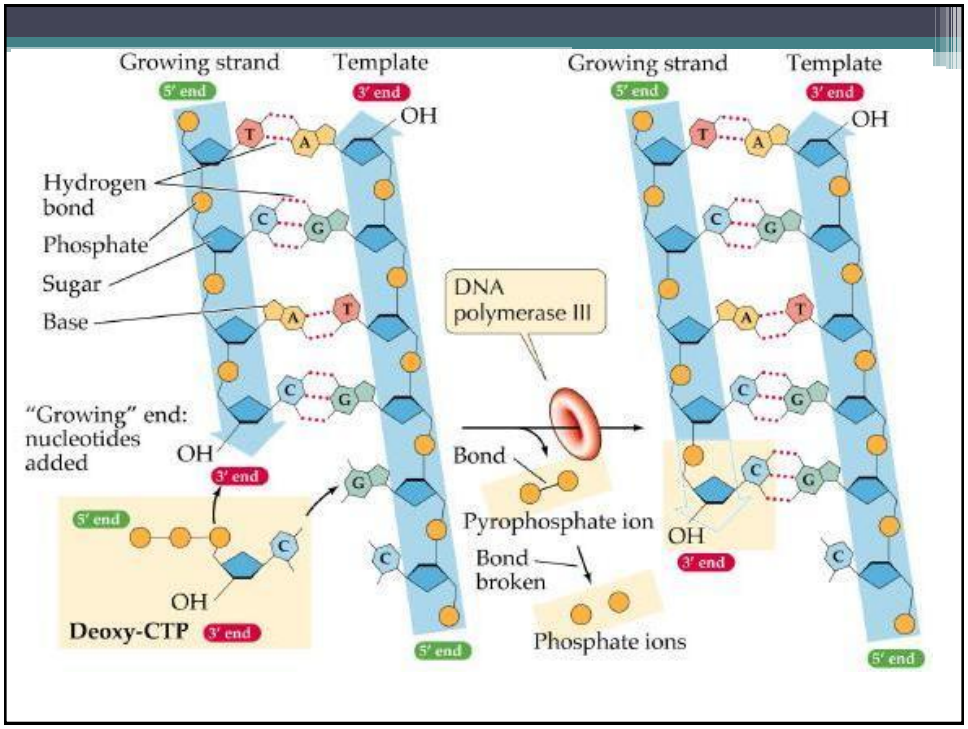
- The helix must undergo local unwinding. Once unwound, the exposed DNA must be stabilized.
- The unwinding and the DNA synthesis increases tension down the helix which must be resolved.
- A primer of some sort must be synthesized, so DNA polymerase can start. This primer is RNA not DNA.
- Once the primers are created synthesis can begin. The two strands employ different methods for replication.
- RNA primers need o be removed prior to the completion of the replication. The gap left needs to be filled with DNA.
- A proofreading mechanism to make sure that correct bases are added.

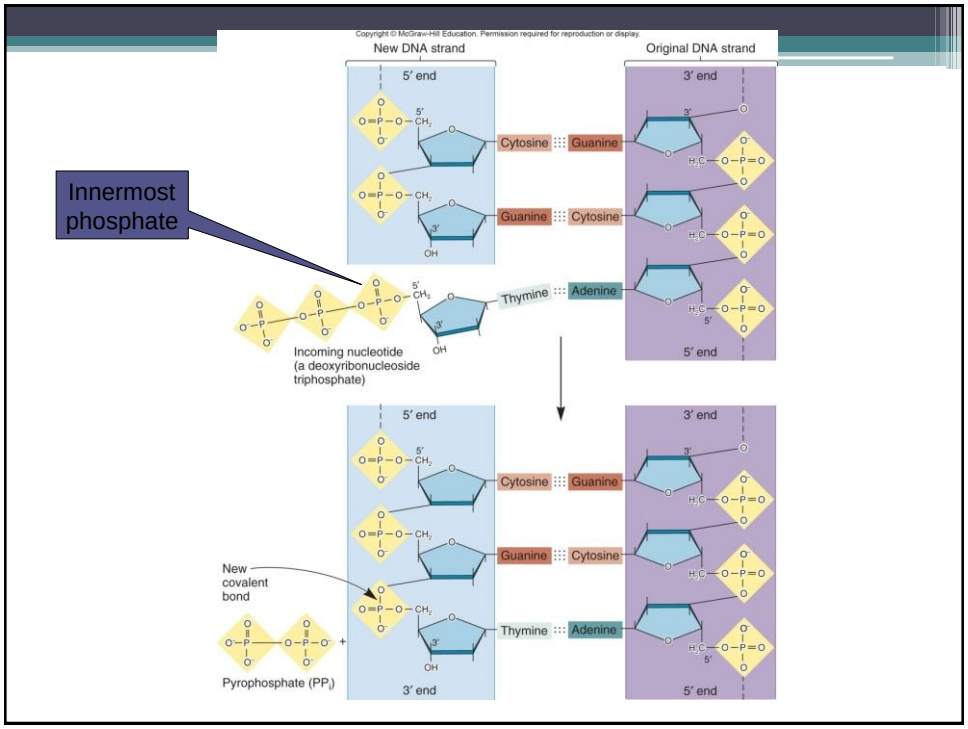




Role of DNA polymerases:

1. Polymerases catalyze the formation of phosphodiester bonds the 3'-OH group of the deoxyribose on the last nucleotide and the 5'-phosphate of the dNTP precursor.
2. DNA polymerase finds the correct complement at each step in the process. 60-90 bases per second in humans.
3. The direction of synthesis is 5' to 3' only.


Growing strand
Template
Growing strand
Template
5′ end
3′ end
OH
Hydrogen bond
Phosphate
Sugar
Base
DNA polymerase III
"Growing" end: nucleotides added
OH
3′ end
5′ end
Bond
Pyrophosphate ion
Bond broken
Phosphate ions
OH
Deoxy-CTP
3′ end
5′ end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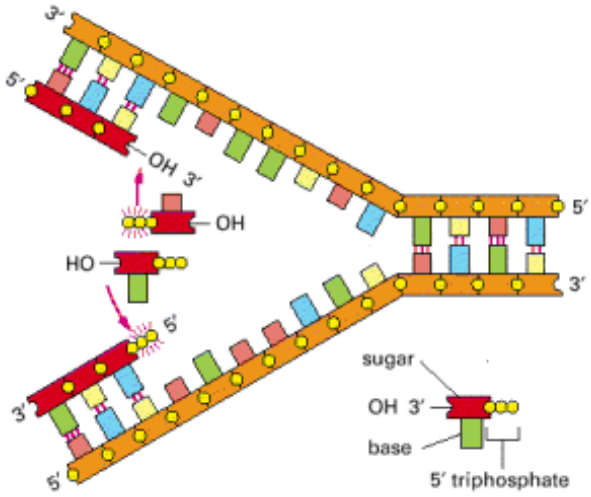
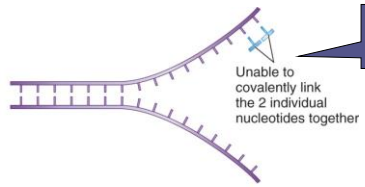
New DNA strand
Original DNA strand
Innermost phosphate
Cytosine
Guanine
Guanine
Cytosine
Thymine
Adenine
Incoming nucleotide (a deoxyribonucleoside triphosphate)
5′ end
3′ end
New covalent bond
Pyrophosphate (PPi)

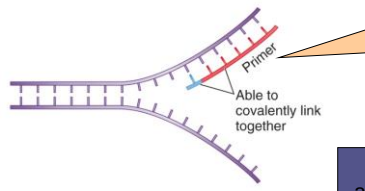
Incorrect model of replication



Unusual features of DNA polymerase function



DNA polymerases cannot initiate DNA synthesis

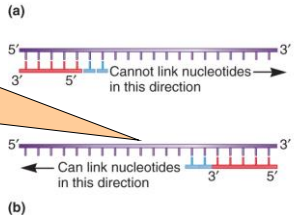


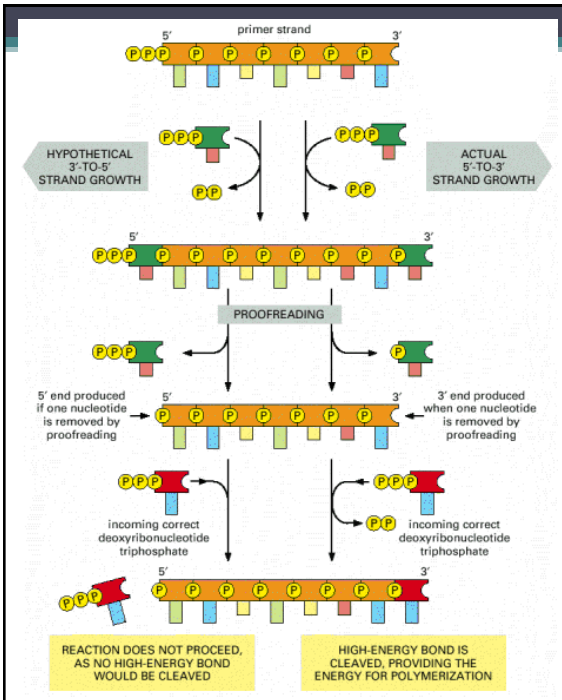
Problem is overcome by the RNA primers synthesized by primase

DNA polymerases can attach nucleotides only in the 5' to 3' direction

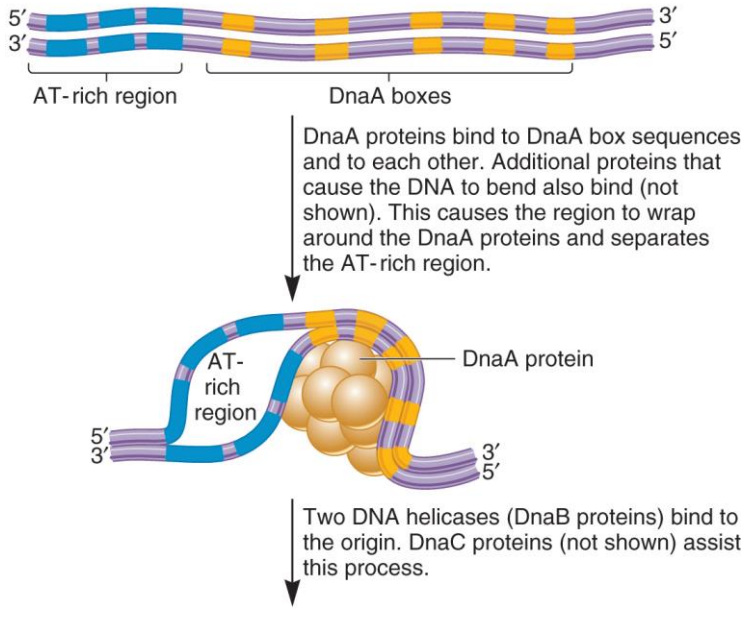
But the two strands are anti-parallel and go in opposite directions

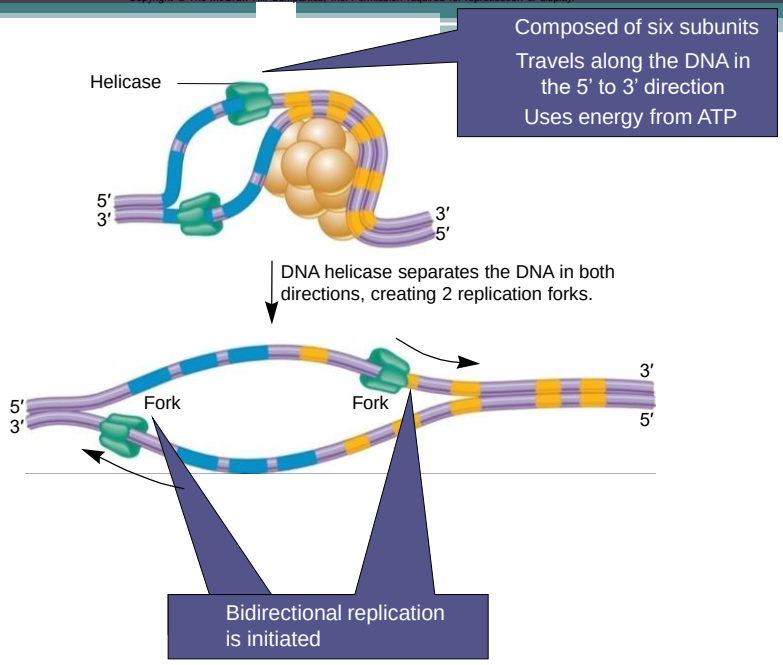
Problem is overcome by synthesizing the new strands both toward, and away from, the replication fork

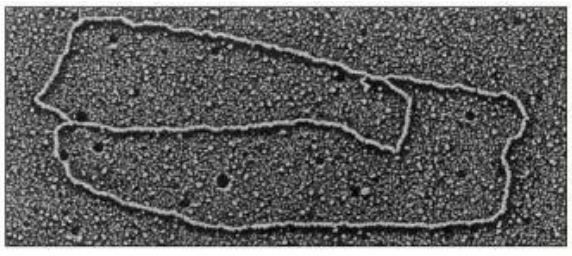


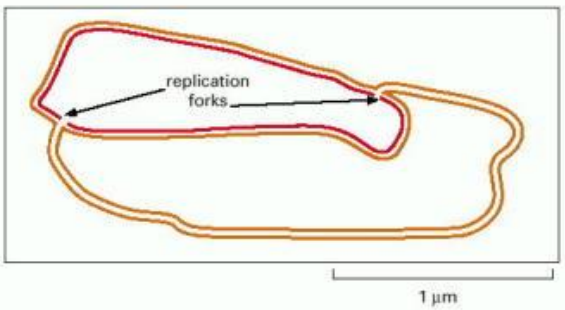


Why 5' to 3'?





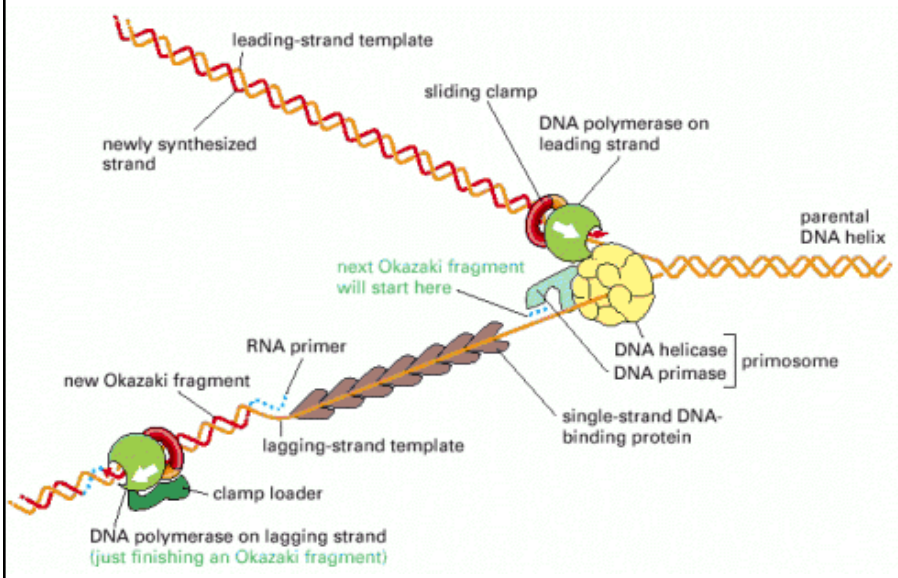


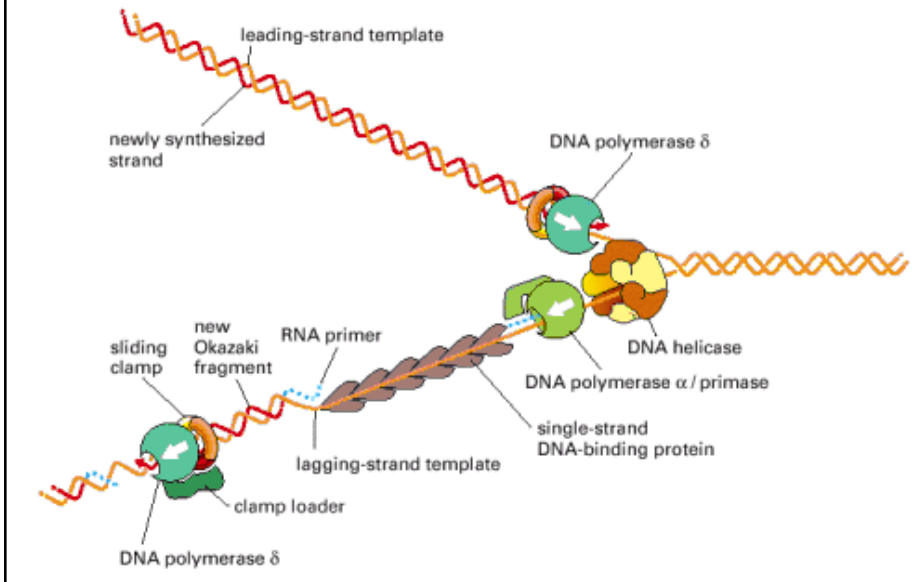


Replication forks

Important enzymes for DNA replication

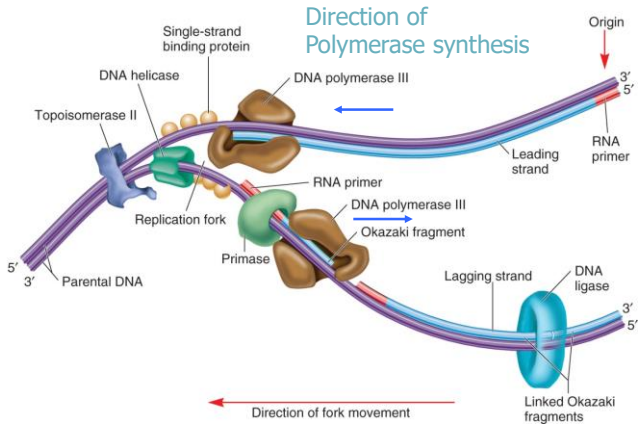
- DNA Helicases
- DNA single-stranded binding proteins
- DNA Topoisomerase
- DNA Polymerase
- Primase
- DNA Ligase



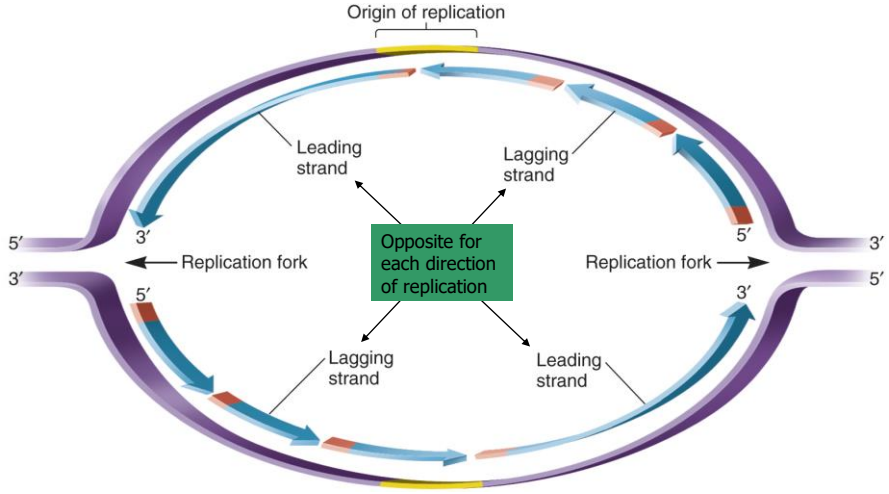


Functions of key proteins involved with bacterial DNA replication

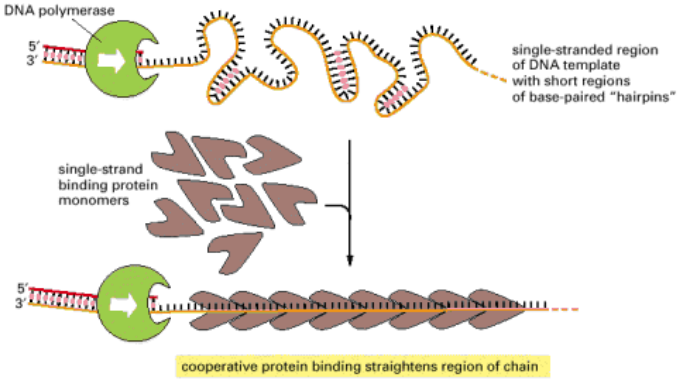
- DNA helicase breaks the hydrogen bonds between the DNA strands.
- Topoisomerase II alleviates positive supercoiling.
- Single-strand binding proteins keep the parental strands apart.
- Primase synthesizes an RNA primer.
- DNA polymerase III synthesizes a daughter strand of DNA.
- DNA polymerase I excises the RNA primers and fills in with DNA (not shown).
- DNA ligase covalently links the Okazaki fragments together.



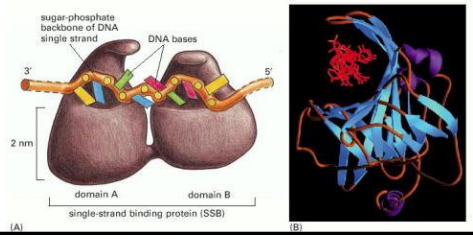
The bidirectional synthesis of leading and lagging strands from a single origin of replication



Single-strand binding proteins (SSBPs)

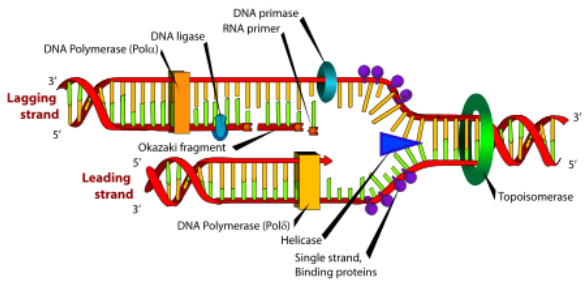


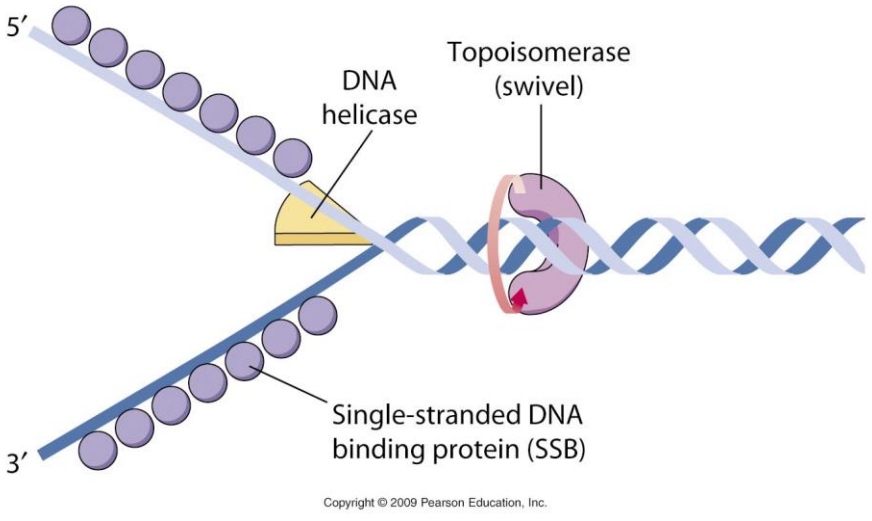
SSBPs bind to the single-strand DNA created by helicase as a tetramer and stabilize it. Replication is 100 times faster when these proteins are bound to the ssDNA.



DNA topoisomerases

- This enzyme catalyzes the formation of negative supercoils that is thought to aid with the unwinding process also.
- There are different members of this family that take part in DNA replication. These include DNA topoisomerase I and II and DNA gyrase.

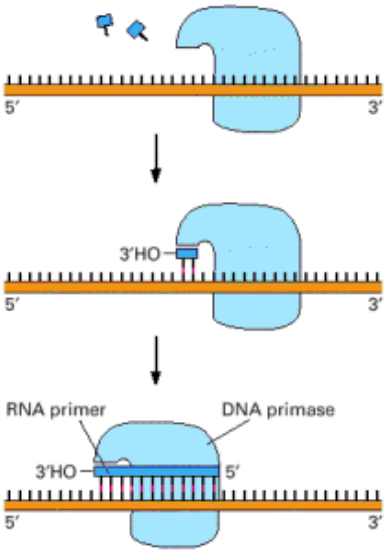




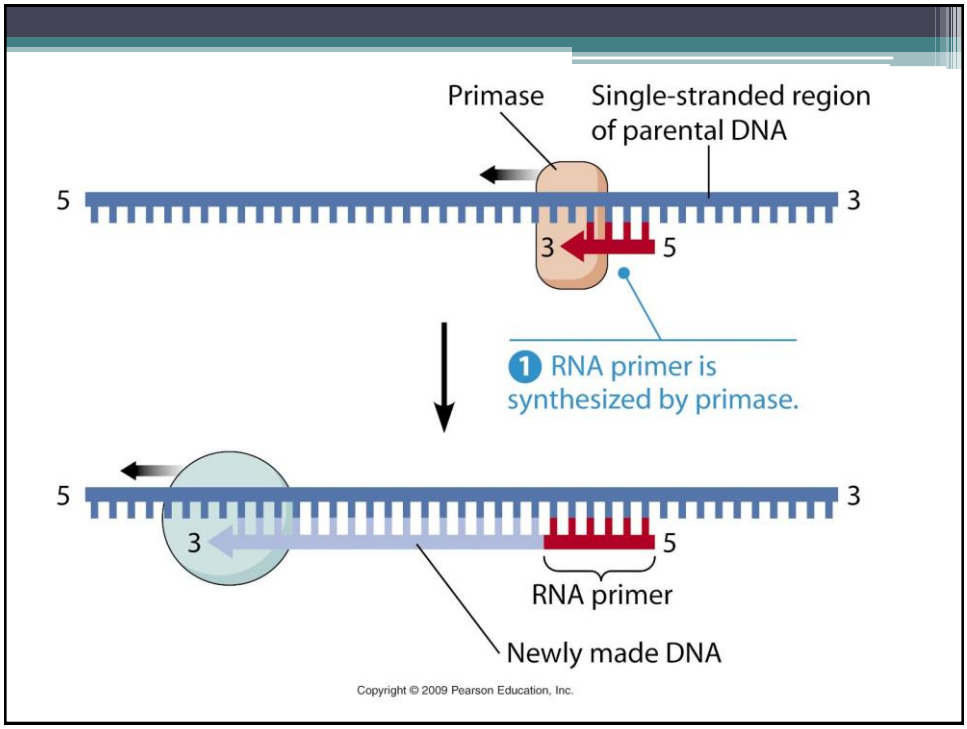
Three main activities of DNA Pol

- 5' to 3' elongation (polymerase activity)
- 3' to 5' exonuclease (proof-reading activity) Error rate is less than 1 in 10^8
- 5' to 3' exonuclease (repair activity)
- The second two activities of DNA Pol I are important for replication, but DNA Polymerase III is the enzyme that performs the 5'→3' polymerase function.

DNA 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.


Primase
Single-stranded region of parental DNA
5
3
3
5
1 RNA primer is synthesized by primase.
5
3
3
5
RNA primer
Newly made DNA
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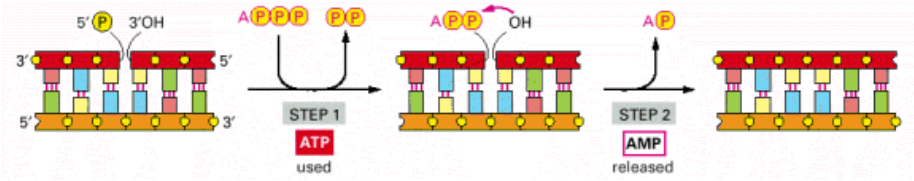
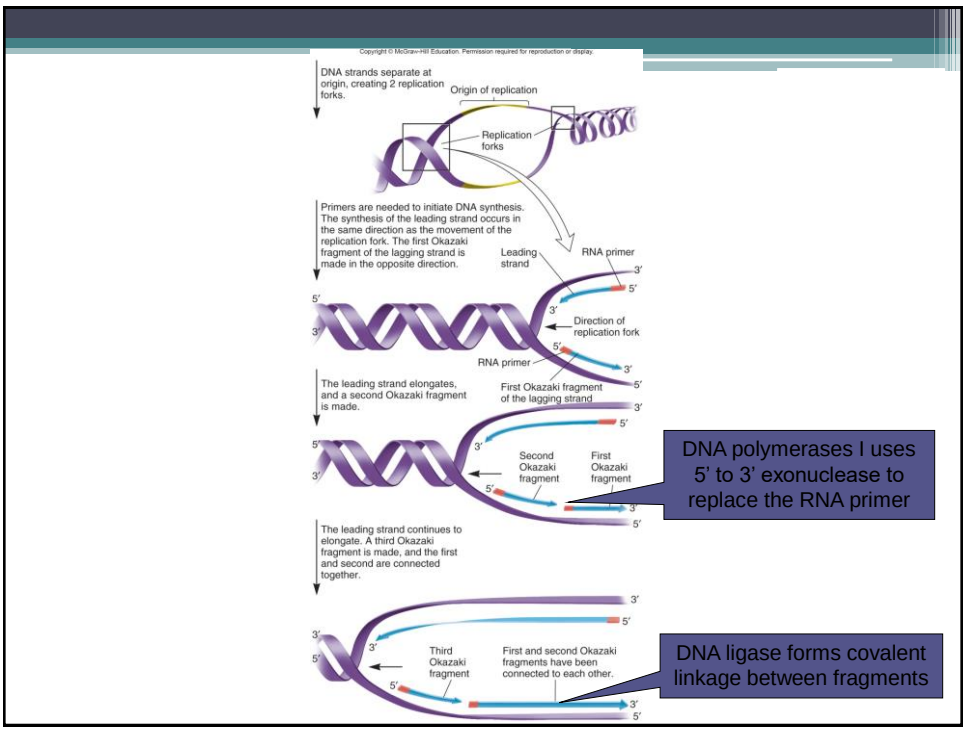
DNA ligase

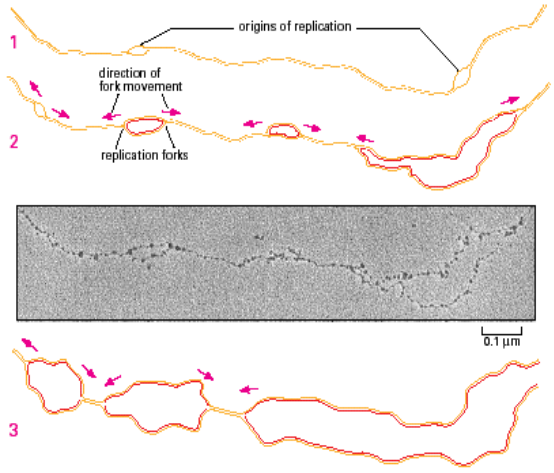

5′ P 3′OH
3′
5′
5′
3′
A P P P P P
STEP 1
ATP
used
A P P OH
A P
STEP 2
AMP
released

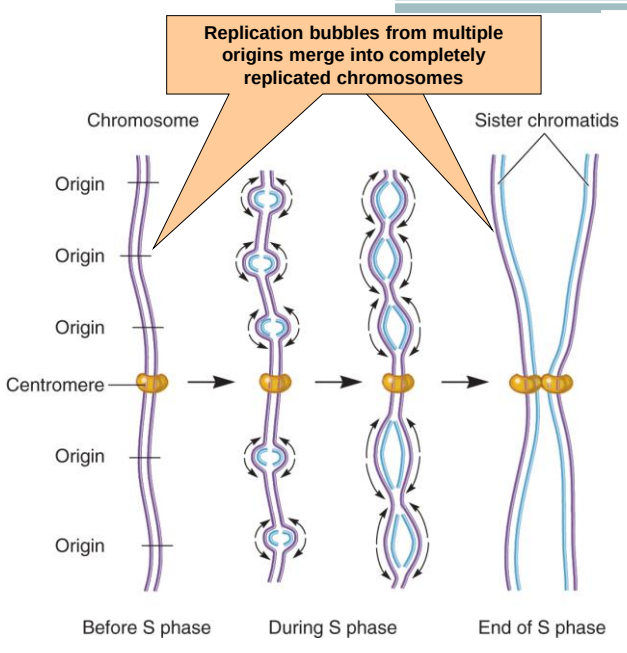
Things to do during replication

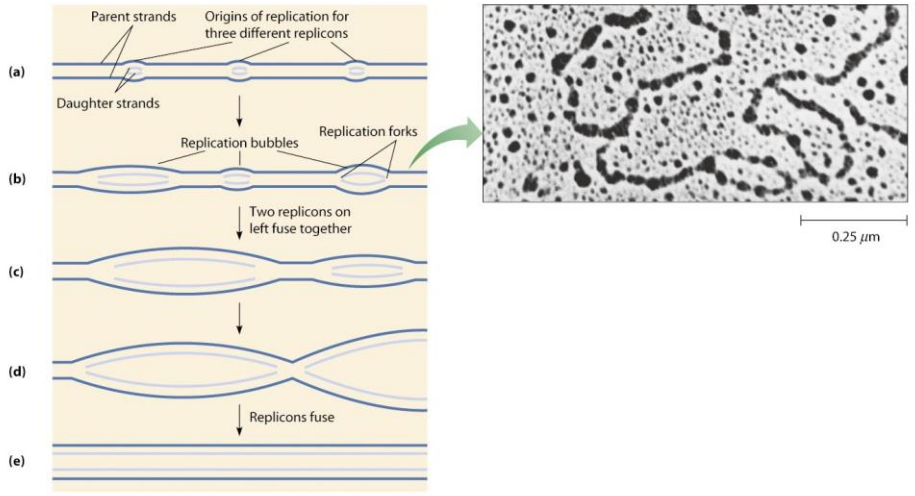
- The helix must undergo local unwinding. Once unwound, the exposed DNA must be stabilized. (by the action of DNA gyrase, DNA helicase and the single-stranded DNA binding proteins).
- The unwinding and the DNA synthesis increases tension down the helix which must be resolved. (by toposiomerases)
- A primer of some sort must be synthesized, so DNA polymerase can start. This primer is RNA not DNA. (RNA primers are synthesized, and the free 3'OH of the primer is used to begin replication).
- Once the primers are created synthesis can begin. The two strands employ different methods for replication. (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)
- RNA primers need to be removed prior to the completion of the replication. (The final product does not have RNA stretches in it. These are removed by the 5' to 3' exonuclease action of Polymerase I). The gap left needs to be filled with DNA. (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)
- DNA polymerase does not have the ability to form the final bond. This is done by the enzyme DNA ligase.
- A proofreading mechanism to make sure that correct bases are added. (done by DNA polymerase III)



Multiple Replication Bubbles during Eukaryotic DNA Replication







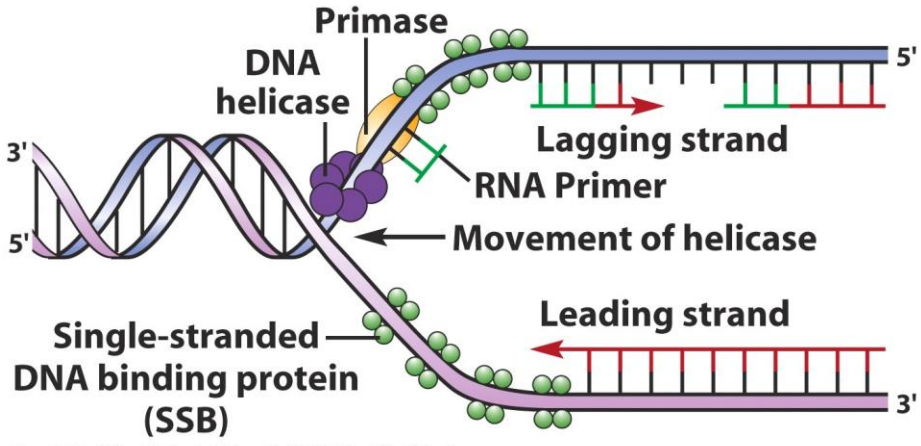
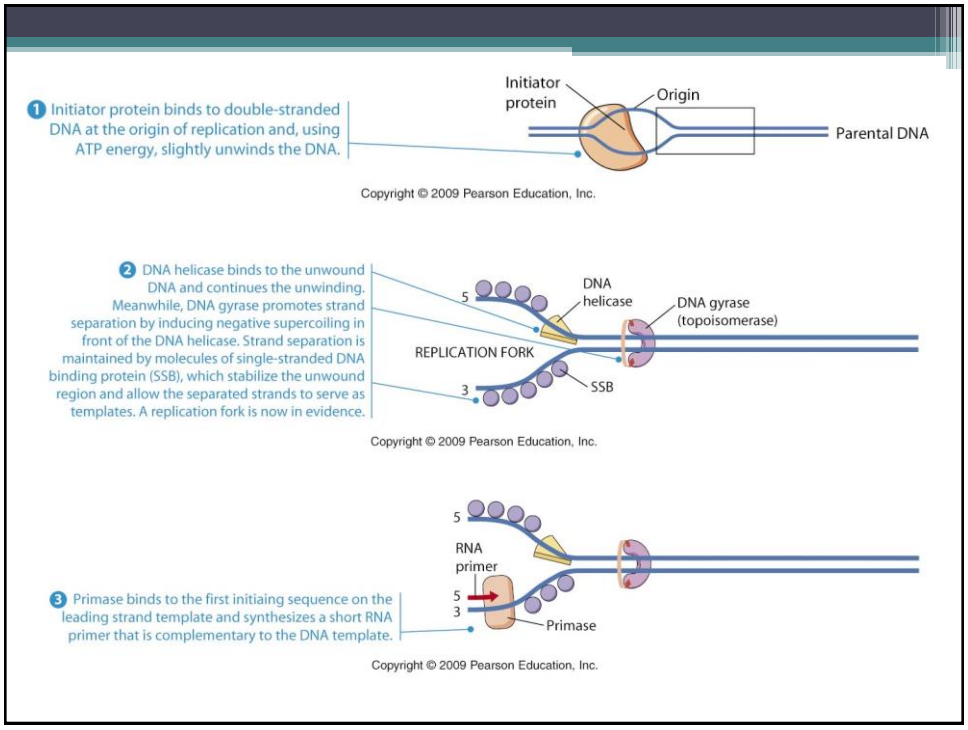


Figure 13-12a Cell and Molecular Biology, 4/e (© 2005 John Wiley & Sons)


1 Initiator protein binds to double-stranded DNA at the origin of replication and, using ATP energy, slightly unwinds the DNA.
Initiator protein
Origin
Parental DNA
Copyright © 2009 Pearson Education, Inc.
2 DNA helicase binds to the unwound DNA and continues the unwinding. Meanwhile, DNA gyrase promotes strand separation by inducing negative supercoiling in front of the DNA helicase. Strand separation is maintained by molecules of single-stranded DNA binding protein (SSB), which stabilize the unwound region and allow the separated strands to serve as templates. A replication fork is now in evidence.
5
3
DNA helicase
DNA gyrase (topoisomerase)
REPLICATION FORK
SSB
Copyright © 2009 Pearson Education, Inc.
3 Primase binds to the first initiaing sequence on the leading strand template and synthesizes a short RNA primer that is complementary to the DNA template.
5
RNA primer
5
3
Primase
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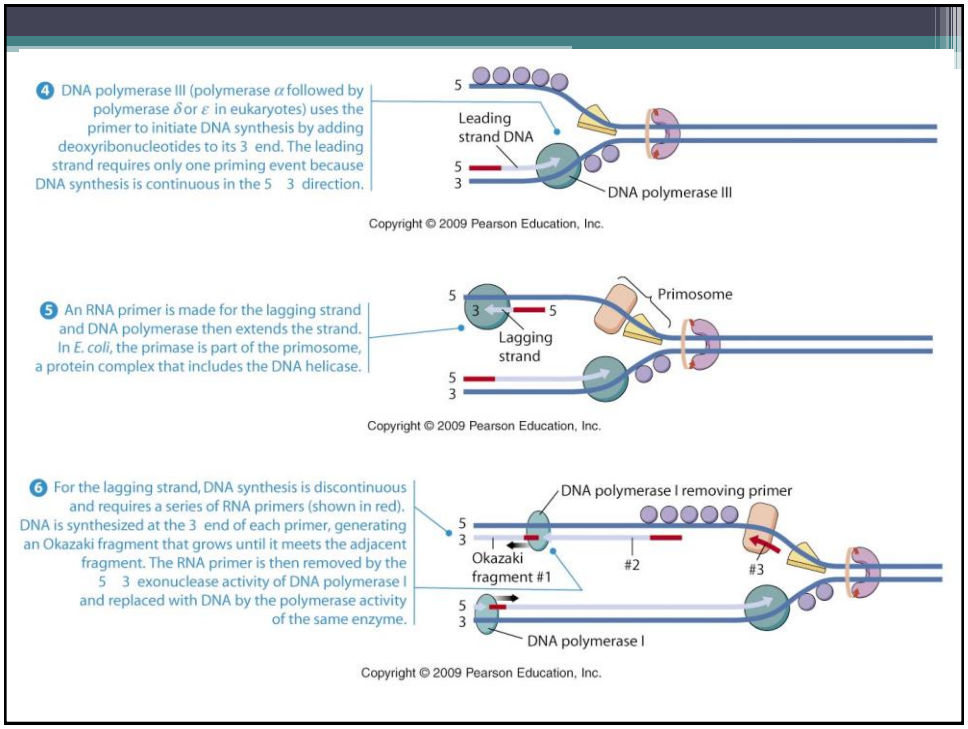
4 DNA polymerase III (polymerase α followed by polymerase δ or ε in eukaryotes) uses the primer to initiate DNA synthesis by adding deoxyribonucleotides to its 3 end. The leading strand requires only one priming event because DNA synthesis is continuous in the 5 3 direction.
5
Leading strand DNA
5
3
DNA polymerase III
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5 An RNA primer is made for the lagging strand and DNA polymerase then extends the strand. In E. coli, the primase is part of the primosome, a protein complex that includes the DNA helicase.
5
3
5
Primosome
Lagging strand
5
3
Copyright © 2009 Pearson Education, Inc.
6 For the lagging strand, DNA synthesis is discontinuous and requires a series of RNA primers (shown in red). DNA is synthesized at the 3 end of each primer, generating an Okazaki fragment that grows until it meets the adjacent fragment. The RNA primer is then removed by the 5 3 exonuclease activity of DNA polymerase I and replaced with DNA by the polymerase activity of the same enzyme.
DNA polymerase I removing primer
5
3
Okazaki fragment #1
#2
#3
5
3
DNA polymerase I
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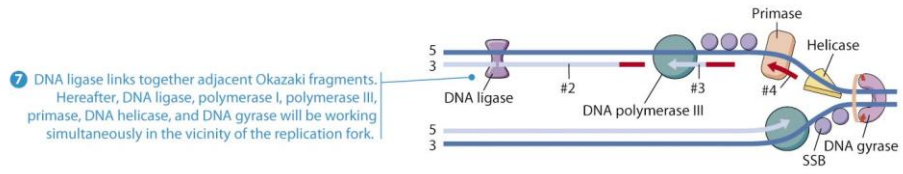
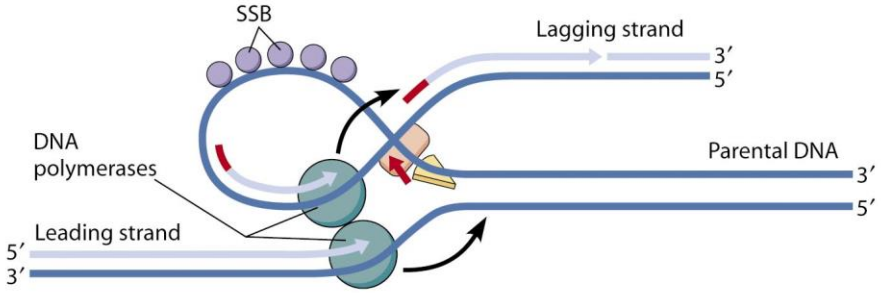
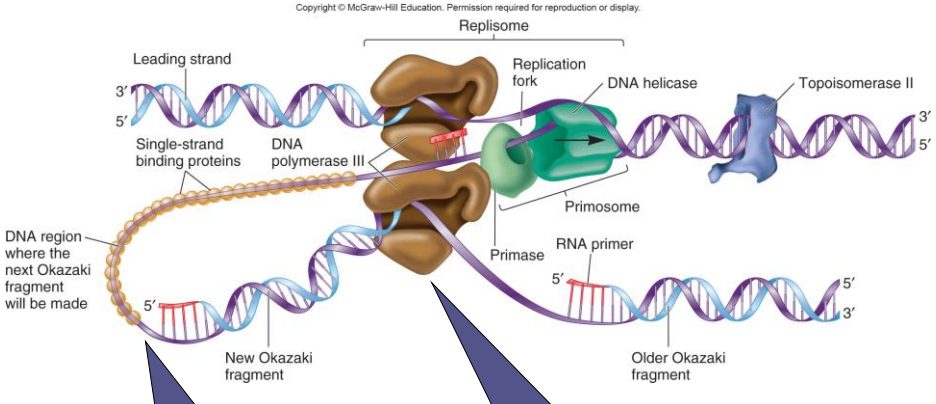
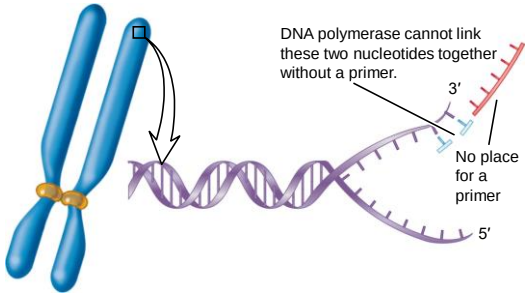




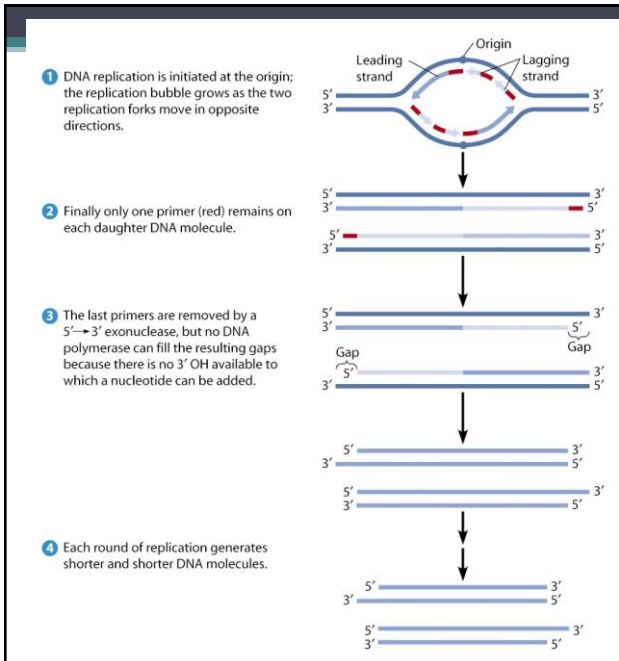
Leading and lagging strand synthesis are linked by the replisome structure



- DNA polymerases possess two unusual features
  - 1. They synthesize DNA only in the 5’ to 3’ direction
  - 2. They cannot initiate DNA synthesis
- These two features pose a problem at the 3’ ends of linear chromosomes-the end of the strand cannot be replicated!



- Therefore if this problem is not solved
  - The linear chromosome becomes progressively shorter with each round of DNA replication
- Indeed, the cell solves this problem by adding DNA sequences to the ends of telomeres
- This requires a specialized mechanism catalyzed by the enzyme telomerase
- Telomerase contains protein and RNA
  - The RNA is complementary to the DNA sequence found in the telomeric repeat
    - This allows the telomerase to bind to the 3’ overhang



Problems arise at the end of chromosomes.

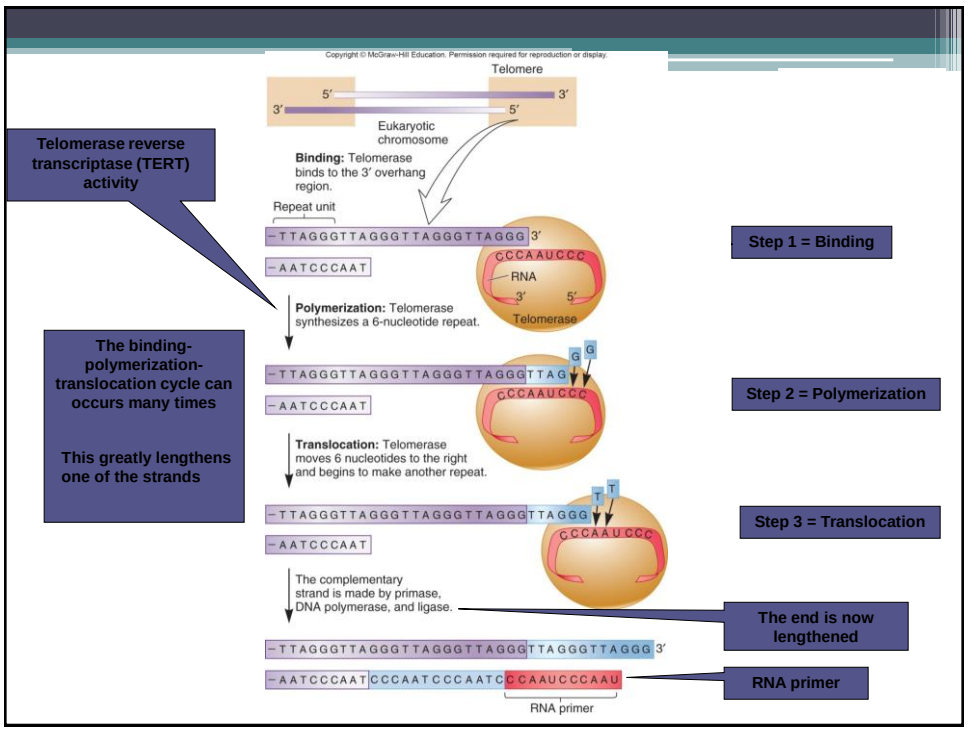


Table 19-1 Some Important DNA Replication Proteins in Bacteria and Eukaryotes

Protein	Cell Type	Main Activities and/or Functions
DNA polymerase I	Bacteria	DNA synthesis; 3′ → 5′ exonuclease (for proofreading); 5′ → 3′ exonuclease; removes and replaces RNA primers used in DNA replication (also functions in excision repair of damaged DNA)
DNA polymerase III	Bacteria	DNA synthesis; 3′ → 5′ exonuclease (for proofreading); used in synthesis of both DNA strands
DNA polymerase α (alpha)	Eukaryotes	Nuclear DNA synthesis; forms complex with primase and begins DNA synthesis at the 3′ end of RNA primers for both leading and lagging strands (also functions in DNA repair)
DNA polymerase γ (gamma)	Eukaryotes	Mitochondrial DNA synthesis
DNA polymerase δ (delta)	Eukaryotes	Nuclear DNA synthesis; 3′ → 5′ exonuclease (for proofreading); involved in lagging and leading strand synthesis (also functions in DNA repair)
DNA polymerase ε (epsilon)	Eukaryotes	Nuclear DNA synthesis; 3′ → 5′ exonuclease (for proofreading); thought to be involved in leading and lagging strand synthesis (also functions in DNA repair)
Primase	Both	RNA synthesis; makes RNA oligonucleotides that are used as primers for DNA synthesis
DNA helicase	Both	Unwinds double-stranded DNA
Single-stranded DNA binding protein (SSB)	Both	Binds to single-stranded DNA; stabilizes strands of unwound DNA in an extended configuration that facilitates access by other proteins
DNA topoisomerase (type I and type II)	Both	Makes single-strand cuts (type I) or double-strand cuts (type II) in DNA; induces and/or relaxes DNA supercoiling; can serve as swivel to prevent overwinding ahead of the DNA replication fork; can separate linked DNA circles at the end of DNA replication
DNA gyrase	Bacteria	Type II DNA topoisomerase that serves as a swivel to relax supercoiling ahead of the DNA replication fork in *E. coli*
DNA ligase	Both	Makes covalent bonds to join together adjacent DNA strands, including the Okazaki fragments in lagging strand DNA synthesis and the new and old DNA segments in excision repair of DNA
Initiator proteins	Both	Bind to origin of replication and initiate unwinding of DNA double helix
Telomerase	Eukaryotes	Using an integral RNA molecule as template, synthesizes DNA for extension of telomeres (sequences at ends of chromosomal DNA)