# DNA Replication, Recombination, and Repair

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# Self introduction

## Current positions

- Professor, University of Kentucky College of Medicine, and Tsinghua University
- Associate editor, *DNA Repair*
- Editorial board member: *Journal of Biological chemistry, Cell Research*

## Education

- BS in Biology (1982), Wuhan University
- PhD in Chemistry (1992), Wayne State University
- Postdoc (1992-1995), Duke University

## Research Interest

- DNA repair, genome instability, and cancer
- Trinucleotide repeat instability and neurodegenerative diseasdes

## Representative publications

- Hypermutability and mismatch repair deficiency in RER$^+$ tumor cells. ***Cell*** 75, 1227-1236, 1993.
- Mismatch repair deficiency in phenotypically normal human cells. ***Science*** 268, 738-740, 1995.
- Isolation of an hMSH2-p160 heterodimer that restores DNA mismatch repair to tumor cells. ***Science*** 268, 1909-1912, 1995.
- Reconstitution of human DNA mismatch repair in a purified system. ***Cell*** 122, 693-705, 2005. **(Cover story)**
- Incision-dependent and error-free repair of $(CAG)_n/(CTG)_n$ hairpins in human cell extracts**.** ***Nature Struct Mol Biol***, 16, 869-75. 2009.

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# DNA Replication

# The James Watson and Francis Crick Model

James Watson

Francis Crick

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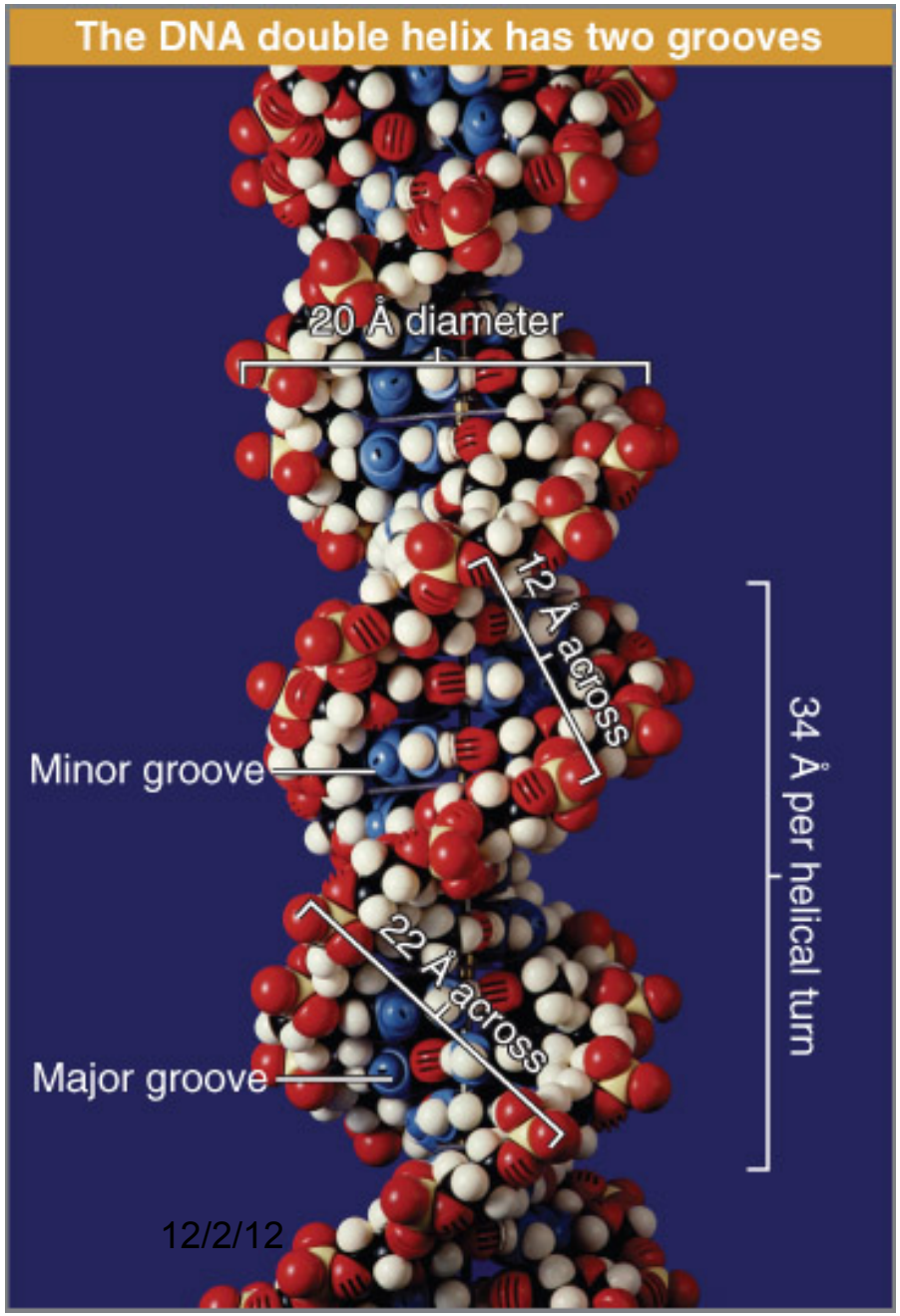
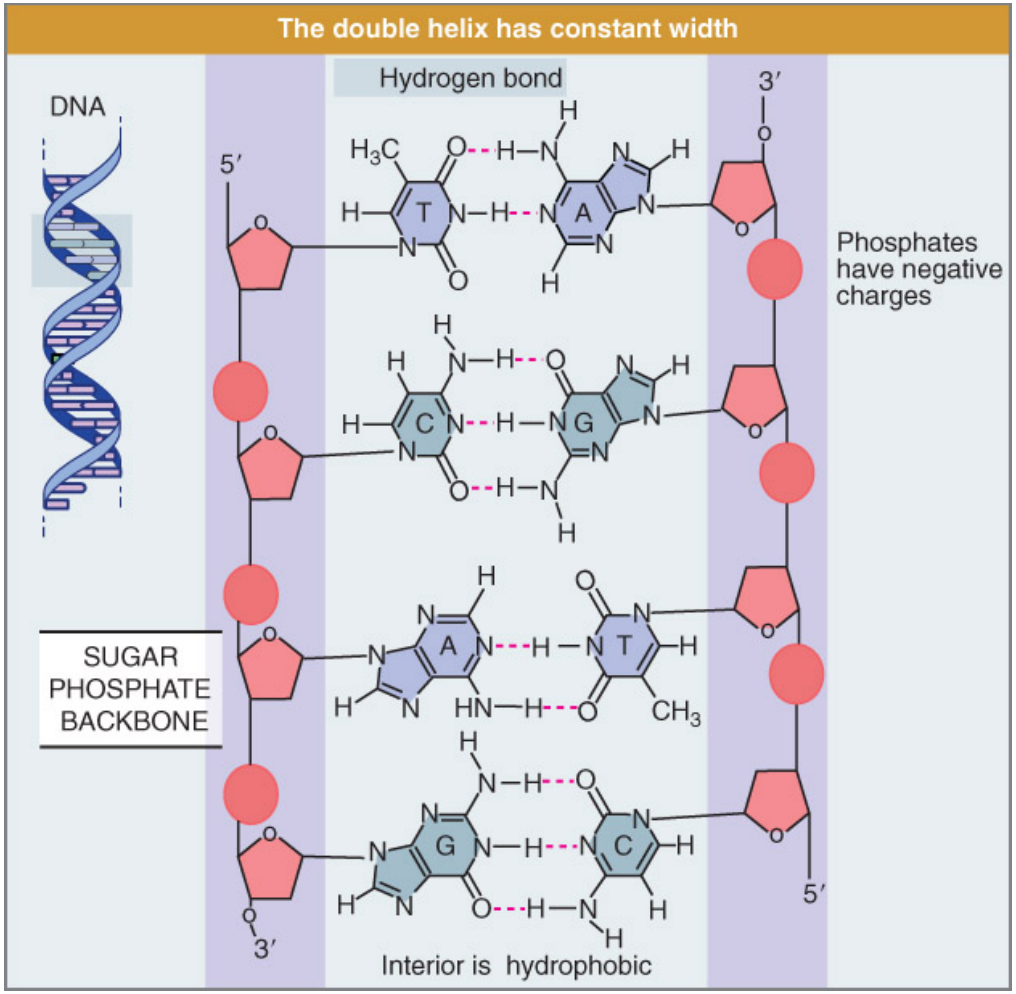


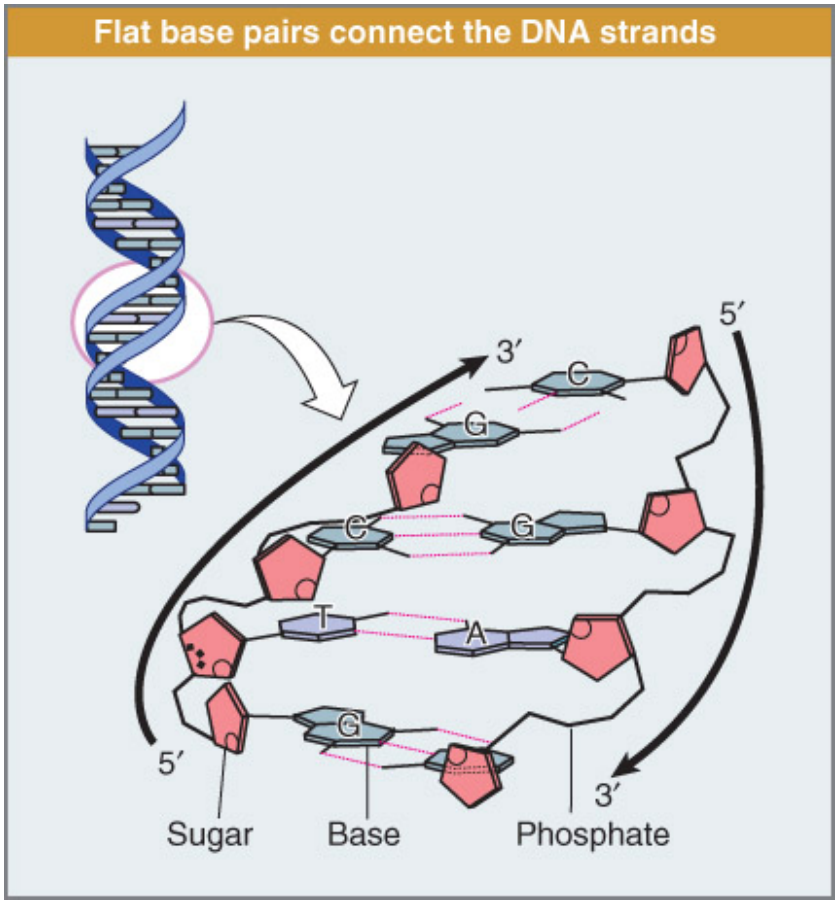

- The diameter of the double helix is 20 Å
  - There is a complete turn every 34 Å
    - Ten base pairs per turn
- The double helix forms:
  - a major (wide) groove
  - a minor (narrow) groove

# DNA Is a Double Helix

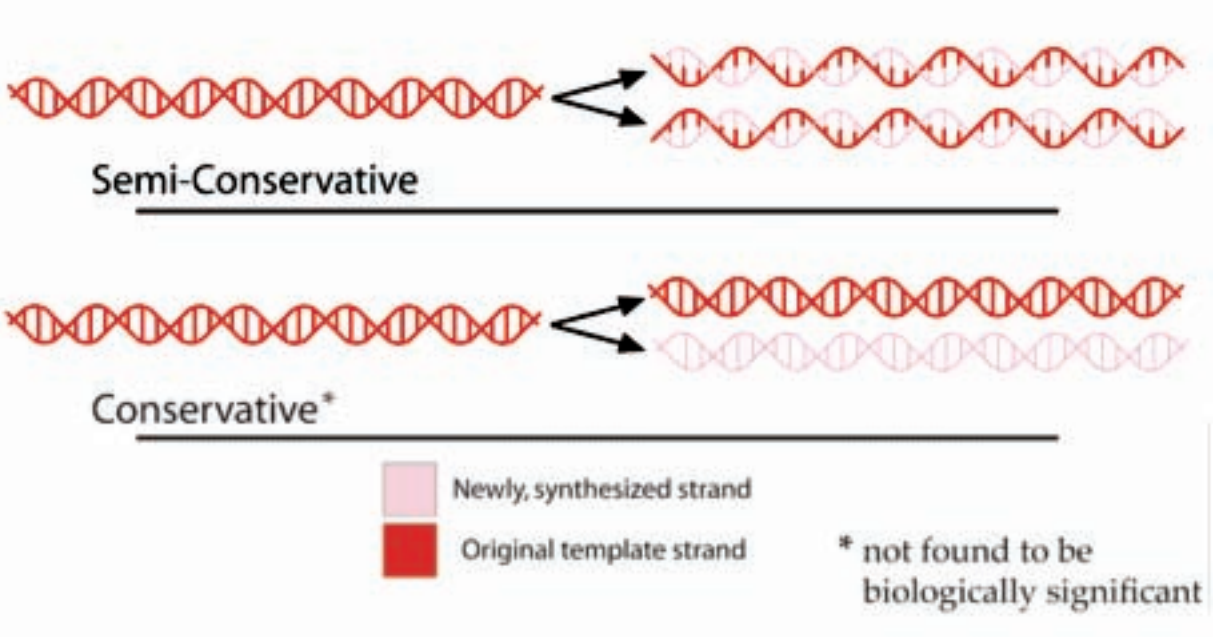
- The B-form of DNA is a double helix consisting of two polynucleotide chains that run anti-parallel.



- The nitrogenous bases of each chain are flat purine or pyrimidine rings
  - They face inward
  - They pair with one another by hydrogen bonding to form A-T or G-C pairs only.



# DNA Replication Hypotheses




The ***semi-conservative*** hypothesis proposed that the two strands of a DNA molecule separate during replication, and each strand then acts as a template for synthesis of a new strand.

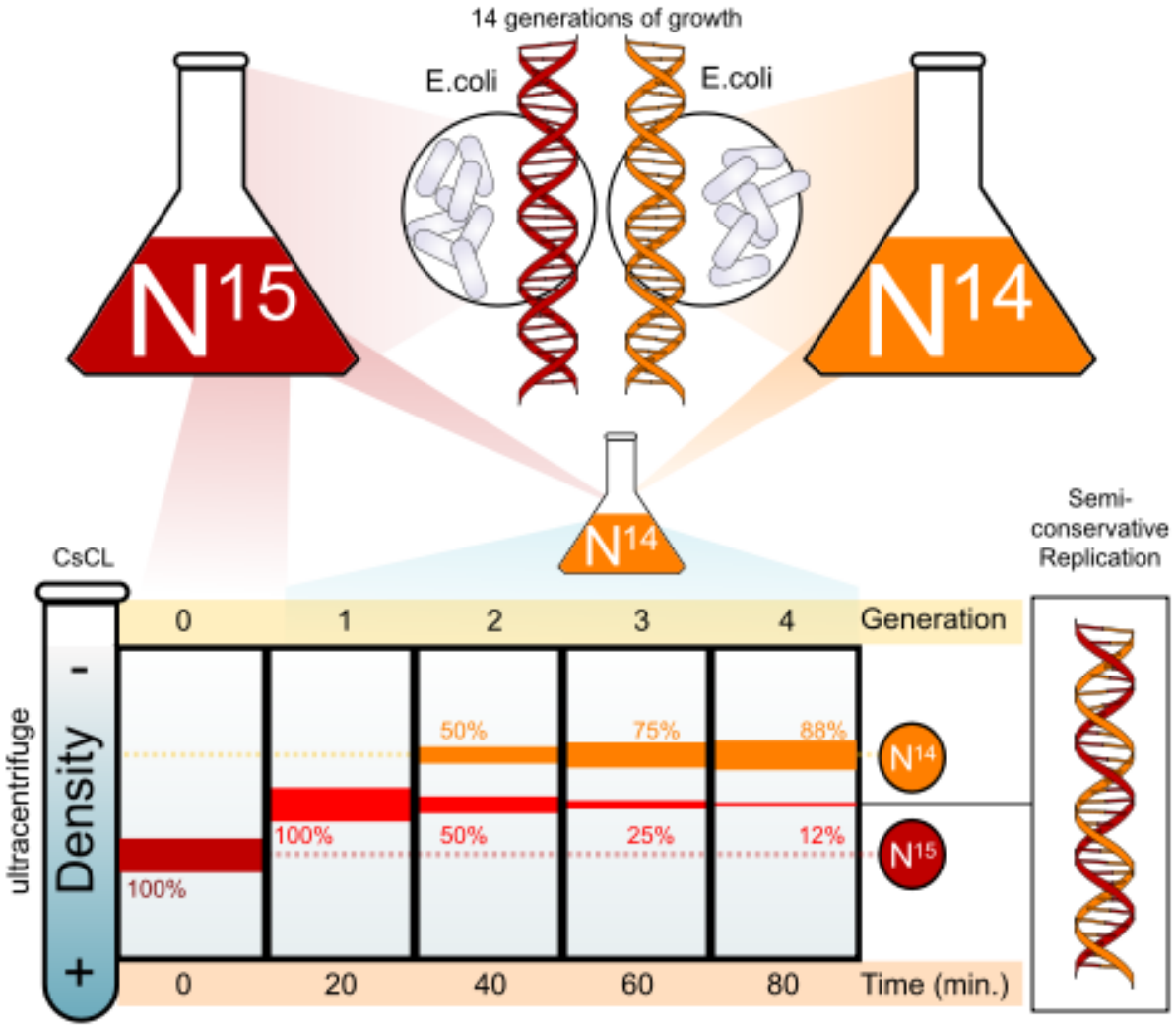
The ***conservative*** hypothesis proposed that the entire DNA molecule acted as a template for synthesis of an entirely new one.

# DNA Replication Is Semiconservative

- The Meselson–Stahl experiment used density labeling to prove that:
  - The single polynucleotide strand is the unit of DNA that is conserved during replication

- Each strand of a DNA duplex acts as a template to synthesize a daughter strand.

# The Meselson–Stahl Experiment

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periment are single polynucleotide chains or even that the DNA molecules studied here correspond to single DNA molecules possessing the structure proposed by Watson and Crick. However, some information has been obtained about the molecules and their subunits; it is summarized below.

- **Meselson M, Stahl FW.**
- **THE REPLICATION OF DNA IN ESCHERICHIA COLI.**
- Proc Natl Acad Sci U S A. 1958 Jul 15;44(7):671-82.

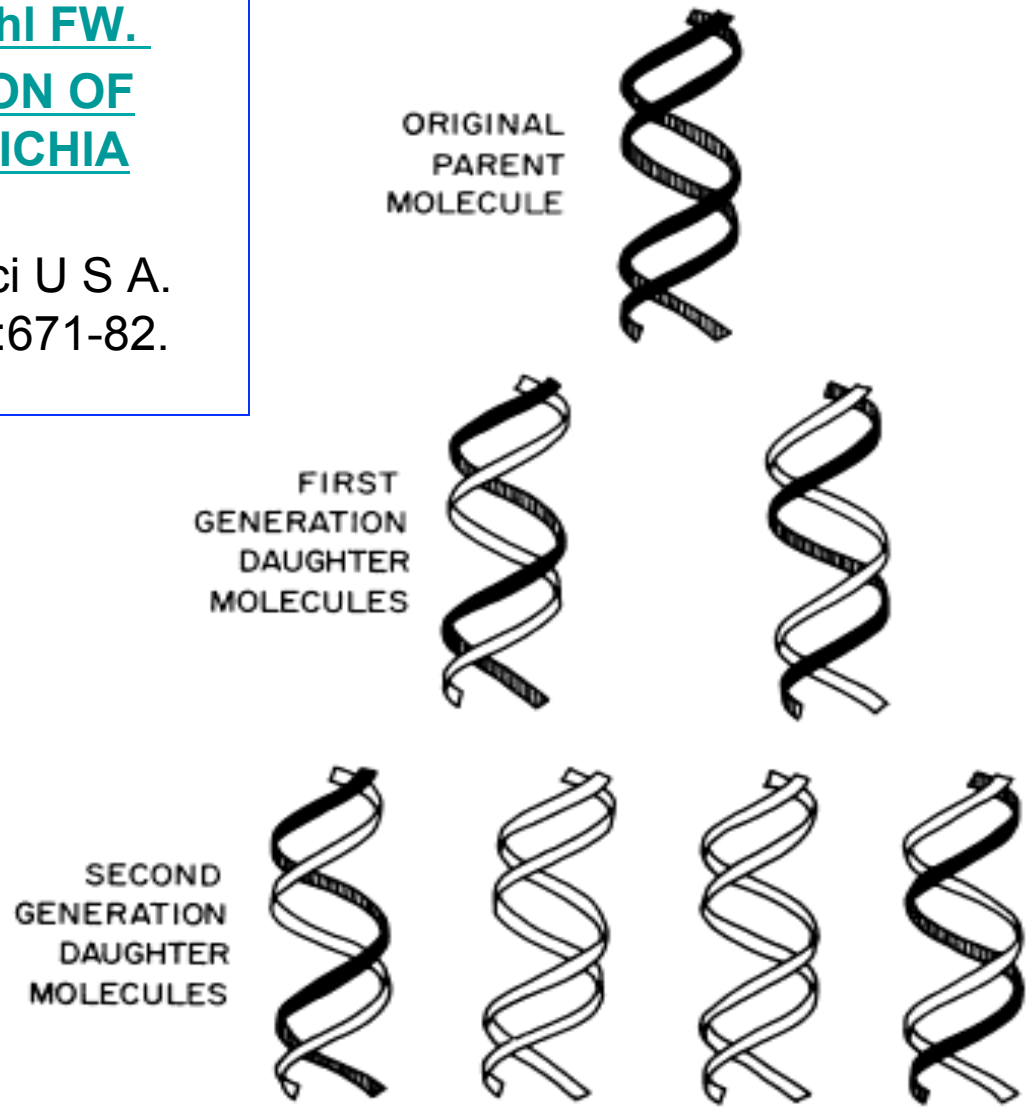
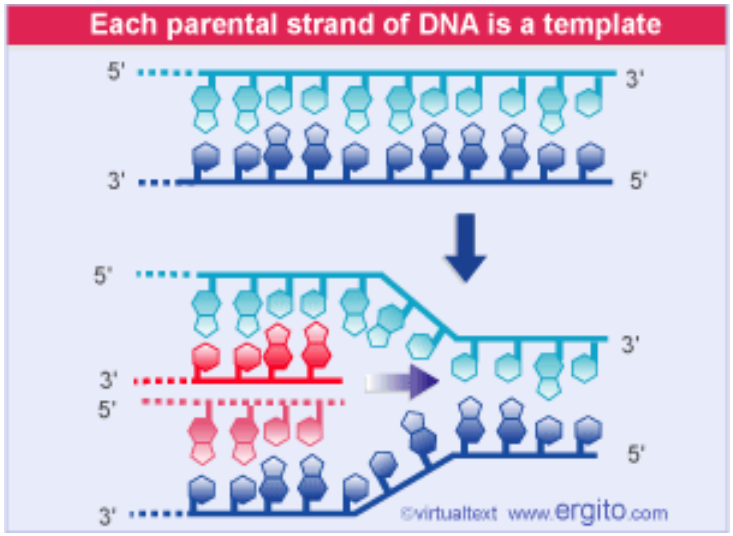



FIG. 6.—Illustration of the mechanism of DNA duplication proposed by Watson and Crick. Each daughter molecule contains one of the parental chains (*black*) paired with one new chain (*white*). Upon continued duplication, the two original

# DNA replication is conducted by DNA polymerases in a semiconservative manner




**Replication** of duplex DNA takes place by synthesis of two new strands that are complementary to the parental strands. The parental duplex is replaced by two identical daughter duplexes, each of which has one parental strand and one newly synthesized strand. Replication is called **semiconservative** because the conserved units are the single strands of the parental duplex.

Replication is conducted by multiple protein complexes, including DNA polymerases.

A **DNA polymerase** is an enzyme that synthesizes a daughter strand(s) of DNA (under direction from a DNA template).

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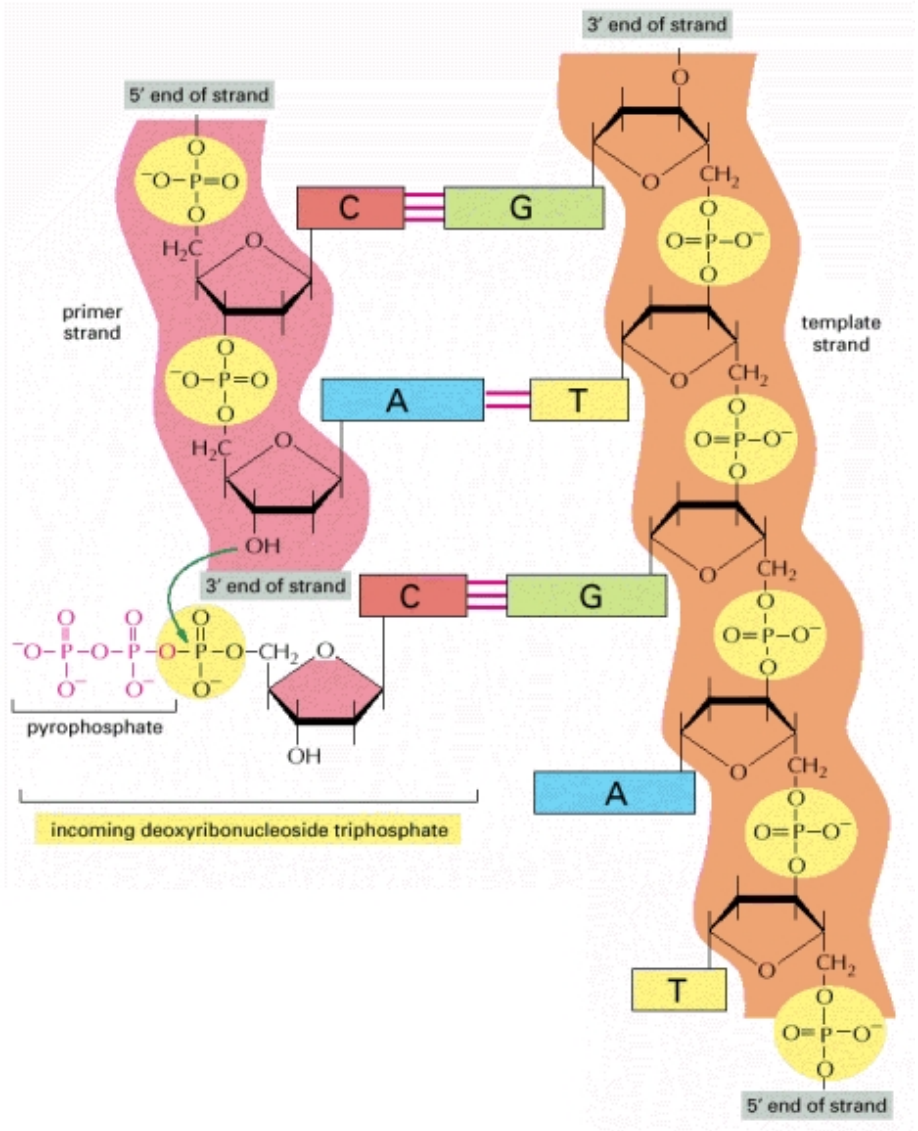
# The chemistry of DNA synthesis




The addition of a deoxyribonucleotide to the 3′ end of a polynucleotide chain (the *primer strand)* is the fundamental reaction by which DNA is synthesized. As shown, base-pairing between an incoming deoxyribonucleoside triphosphate and an existing strand of DNA (the template strand) guilds the formation of the new strand of DNA and causes it to have a complementary nucleotide sequence.

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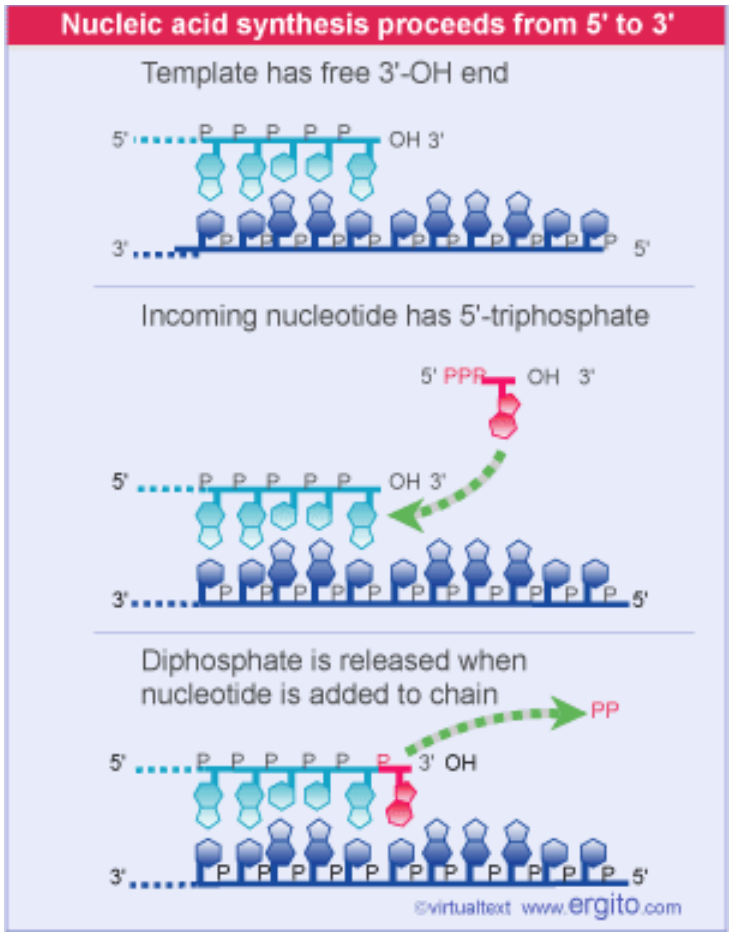
# DNA synthesis occurs only in a 5’ to 3’ orientation




DNA is synthesized by adding nucleotides to the 3’-OH end of the growing chain, so that the new chain grows in the 5’→3’ direction. The precursor for DNA synthesis is a nucleoside triphosphate, which loses the terminal two phosphate groups in the reaction.

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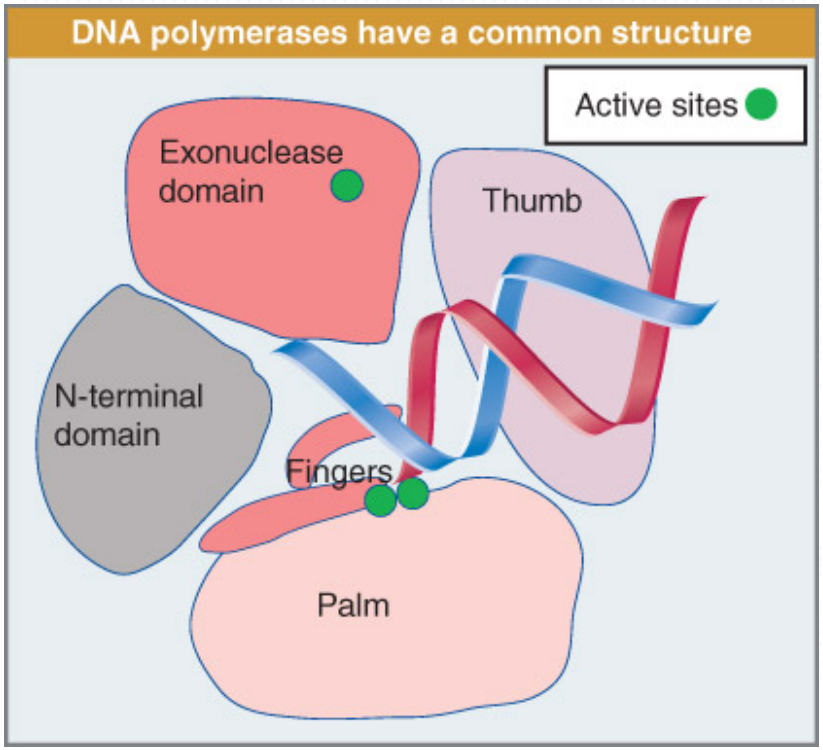
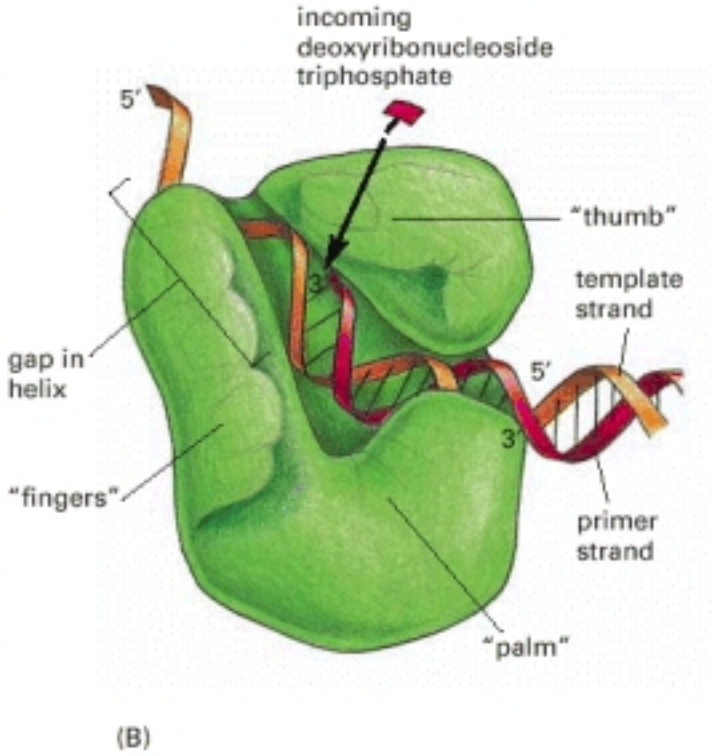
# DNA synthesis catalyzed by DNA polymerase




The common organization of DNA polymerases has a **palm** that contains the catalytic site, **fingers** that position the template, a **thumb** that binds DNA and is important in processivity, **an exonuclease** domain with its own active site, and an **N-terminal domain**.

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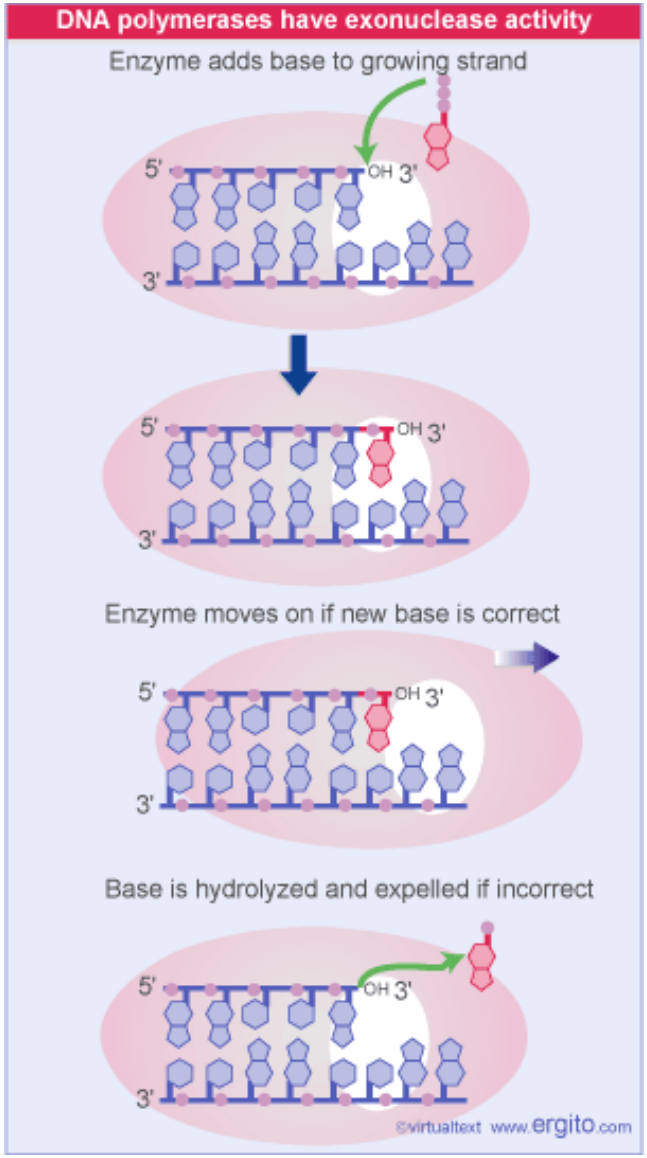
# Properties of DNA polymerases




**Processivity** describes the ability of an enzyme to perform multiple catalytic cycles with a single template instead of dissociating after each cycle.

**Proofreading** refers to any mechanism for correcting errors in protein or nucleic acid synthesis that involves scrutiny of individual units after they have been added to the chain.

DNA polymerases often have a **3' – 5' exonuclease activity** that is used to excise incorrectly paired bases.

**The fidelity** of replication is improved by proofreading by a factor of ~100.

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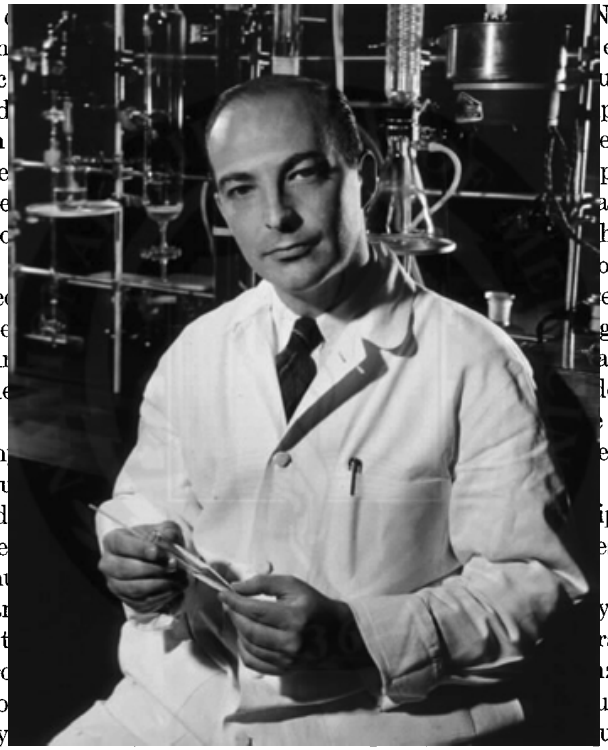
# Arthur Kornberg & DNA Polymerase I

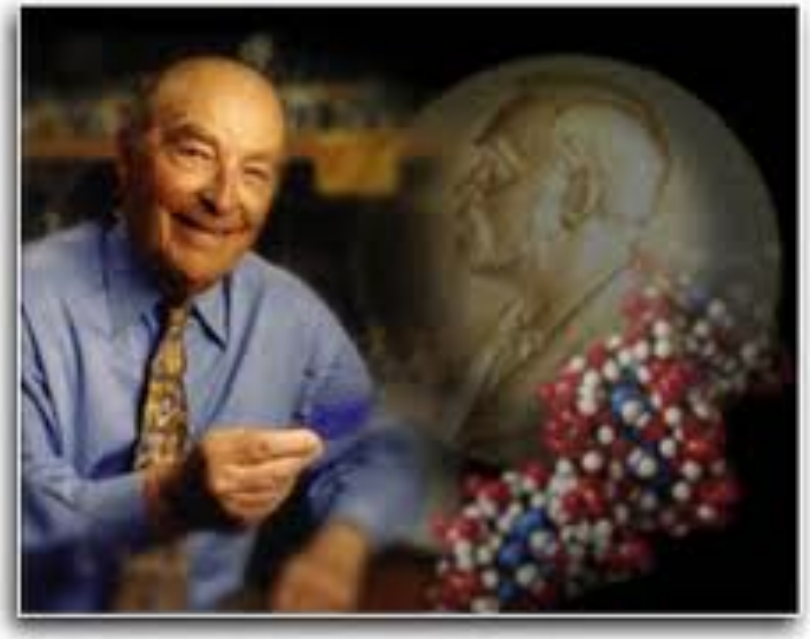
I. R. Lehman,* Maurice J. Bessman,† Ernest S. Simms, and Arthur Kornberg

*From the Department of Microbiology, Washington University School of Medicine, St. Louis, Missouri*

(Received for publication, October 10, 1957)







In addition, polymerized DNA and $Mg^{++}$ were found to be indispensable for the reaction. Deoxynucleoside diphosphates are inert; and as a further indication of the specificity of the enzyme for the triphosphates, the synthesis of DNA is accompanied by a release of inorganic pyrophosphate, and reversal of

cent each of KCl and NaCl. The packed cells were suspended in 6 ml. of alcohol-ether (3:1), incubated at 37° for 30 minutes, centrifuged, and resuspended in 6 ml. of the alcohol-ether solution. After centrifugation, the cells were dried over KOH *in vacuo*. The dry powder was then suspended in 3.0 ml. of 1 N NaOH, and left for 15 hours at 37°. The turbid, viscous solution was chilled to 0° and treated with 1.0 ml. of 5 N perchloric acid. The precipitate, collected by centrifugation, was suspended in 1.75 ml. of water and dissolved by the addition of

# Arthur Kornberg & DNA Polymerase I

- **Arthur Kornberg's Discovery of DNA Polymerase I**
- Nicole Kresge, Robert D. Simoni and Robert L. Hill

- Enzymatic Synthesis of Deoxyribonucleic Acid. I. Preparation of Substrates and Partial Purification of an Enzyme from Escherichia coli (Lehman, I. R., Bessman, M. J., Simms, E. S., and Kornberg, A. (1958) J. Biol. Chem. 233, 163-170)

- Enzymatic Synthesis of Deoxyribonucleic Acid. II. General Properties of the Reaction (Bessman, M. J., Lehman, I. R., Simms, E. S., and Kornberg, A. (1958) J. Biol. Chem. 233, 171-177)

- "These two Classics were **declined** by the JBC when submitted in the fall of 1957. Among the critical comments were: "It is very doubtful that the authors are entitled to speak of the enzymatic synthesis of DNA"; "Polymerase is a poor name"; "Perhaps as important as the elimination of certain banalities..." etc. Through the fortunate intervention of John Edsall, who had just assumed the position of Editor-in-Chief in May 1958, the two papers were eventually accepted and appeared in the July 1958 issue. A more in-depth account of the discovery of DNA polymerase can be found in Lehman's JBC Reflections. One year after these Classics were published, Kornberg was awarded the Nobel Prize in Physiology or Medicine …"

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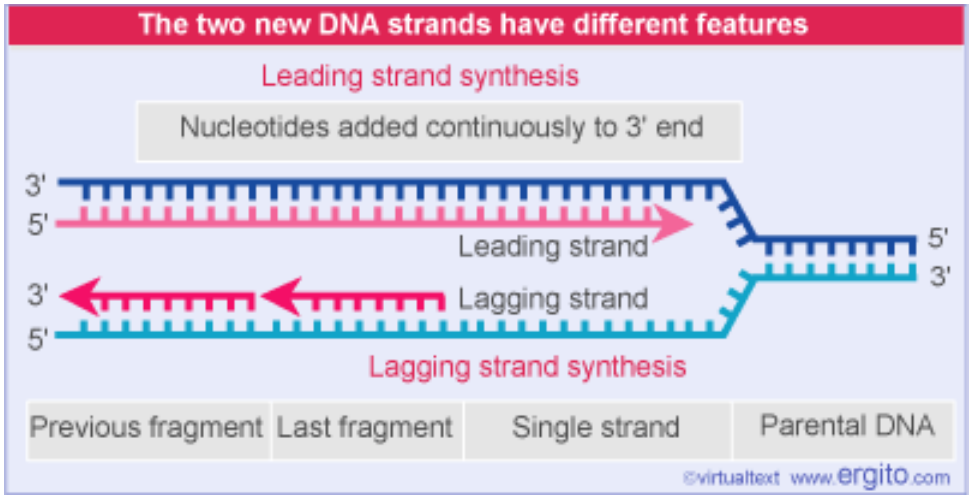
# Semidiscontinuous replication




**The leading strand** of DNA is synthesized continuously in the 5′-3′ direction.

**The lagging strand** of DNA must grow overall in the 3′-5′ direction and is synthesized discontinuously in the form of short fragments (5′-3′) that are later connected covalently.

**Okazaki fragments** are the short stretches of 100-200 bases produced during discontinuous replication; they are later joined into a covalently intact strand.

**Semidiscontinuous replication** is mode in which one new strand is synthesized continuously while the other is synthesized discontinuously.

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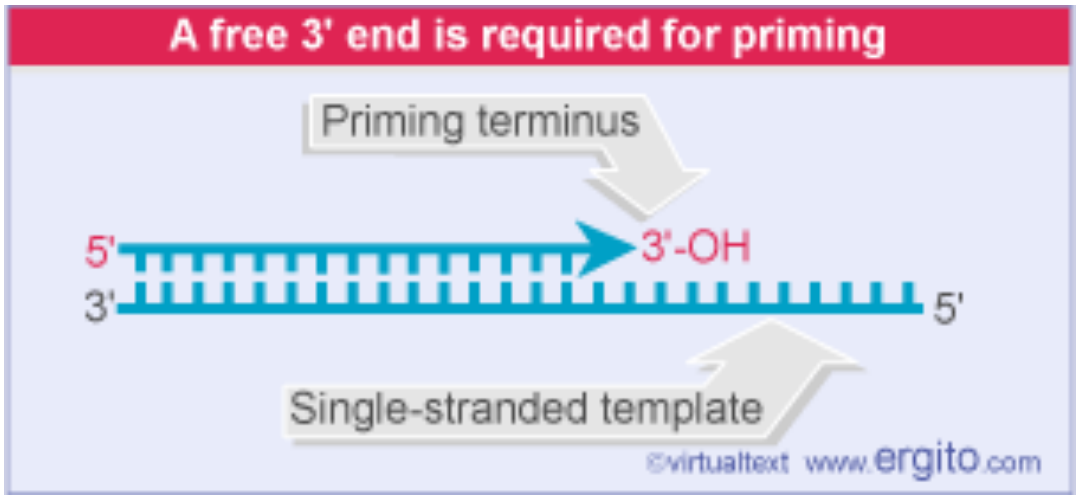
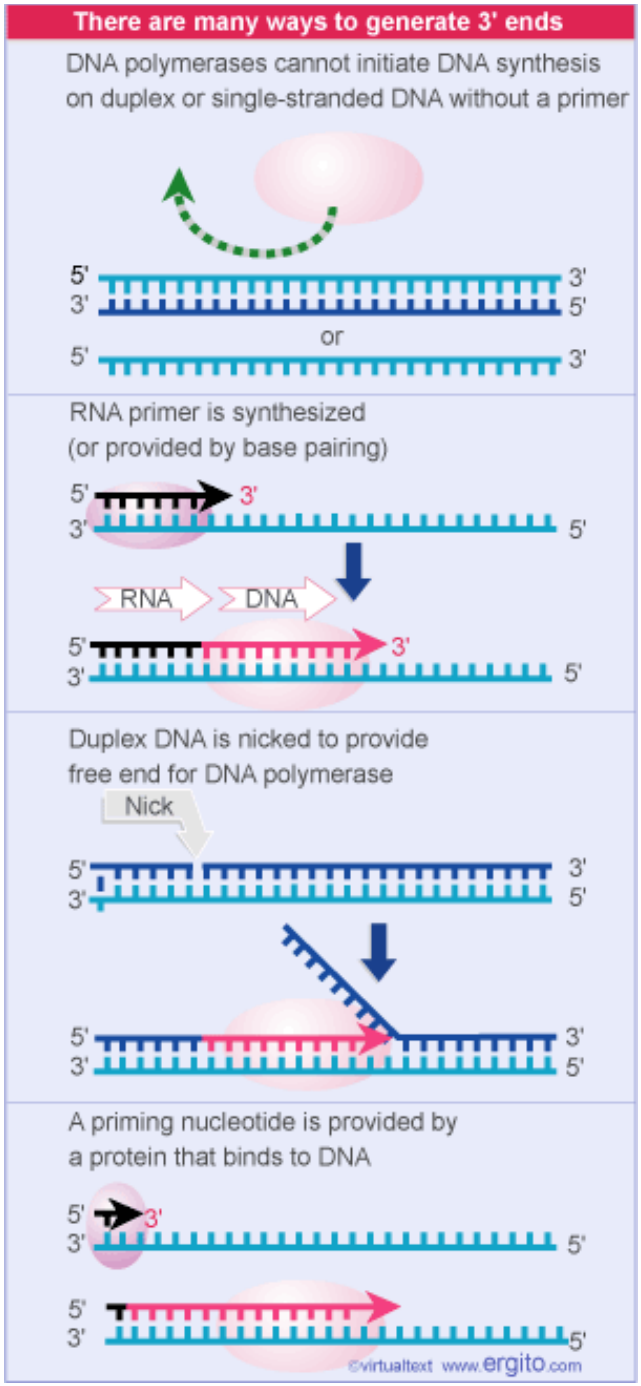
# Priming is required to start DNA synthesis




A **primer** is a short sequence (often of RNA) that is paired with one strand of DNA and provides a free 3′-OH end at which a DNA polymerase starts synthesis of a deoxyribonucleotide chain.

The **primase** is a type of RNA polymerase that synthesizes short segments of RNA that will be used as primers for DNA replication.

All DNA polymerases require a **3′–OH priming end** to initiate DNA synthesis.




There are several methods for providing the free 3’-OH end that DNA polymerases require to initiate DNA synthesis, including **an RNA primer**, **a nick in DNA**, **or a priming protein**.

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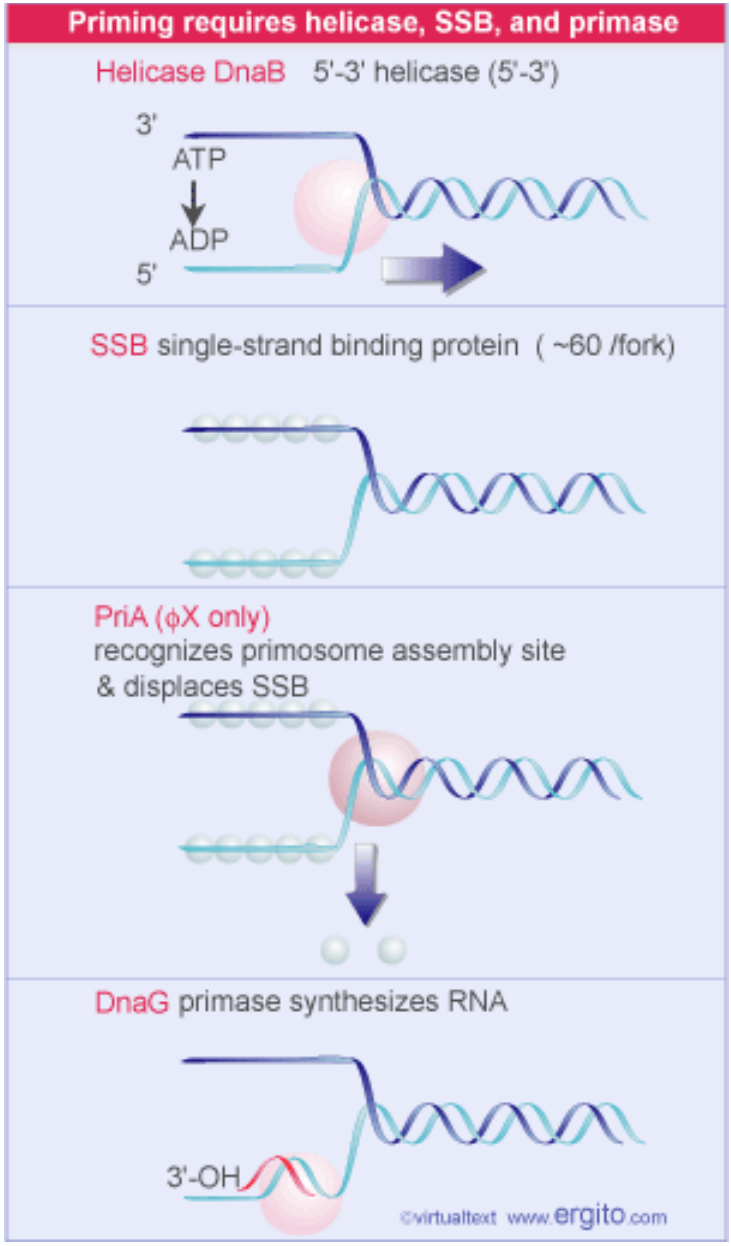


Initiation of DNA replication requires several enzymatic activities, including helicases, single-strand DNA binding proteins, and synthesis of the primer.

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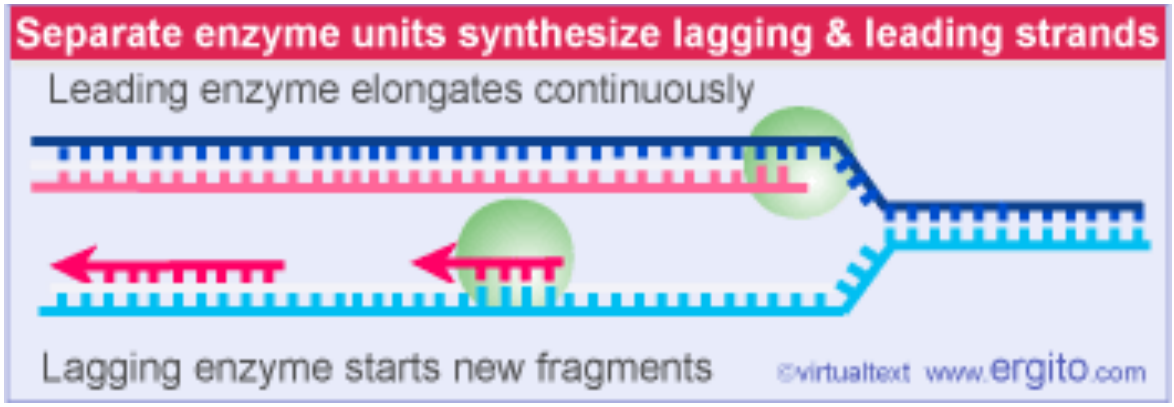
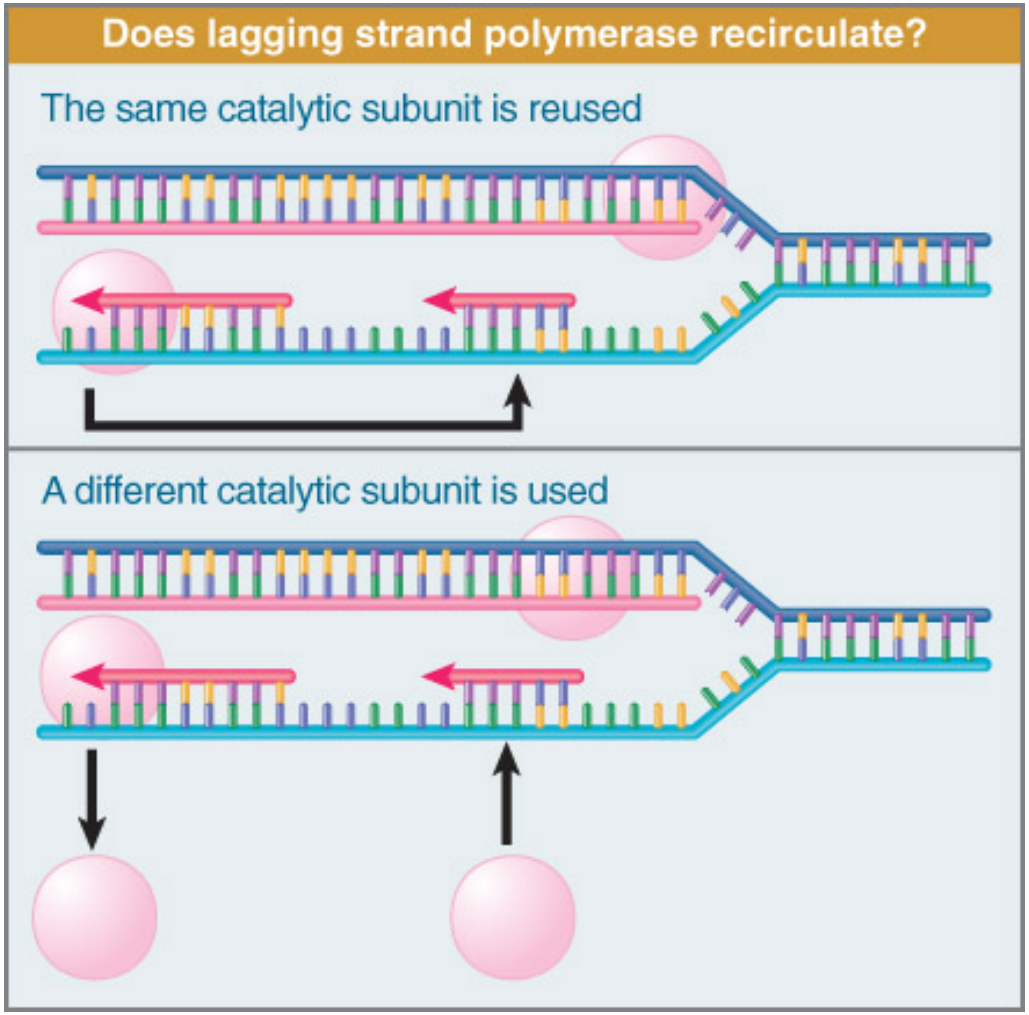
# Coordinating synthesis of the lagging and leading strands

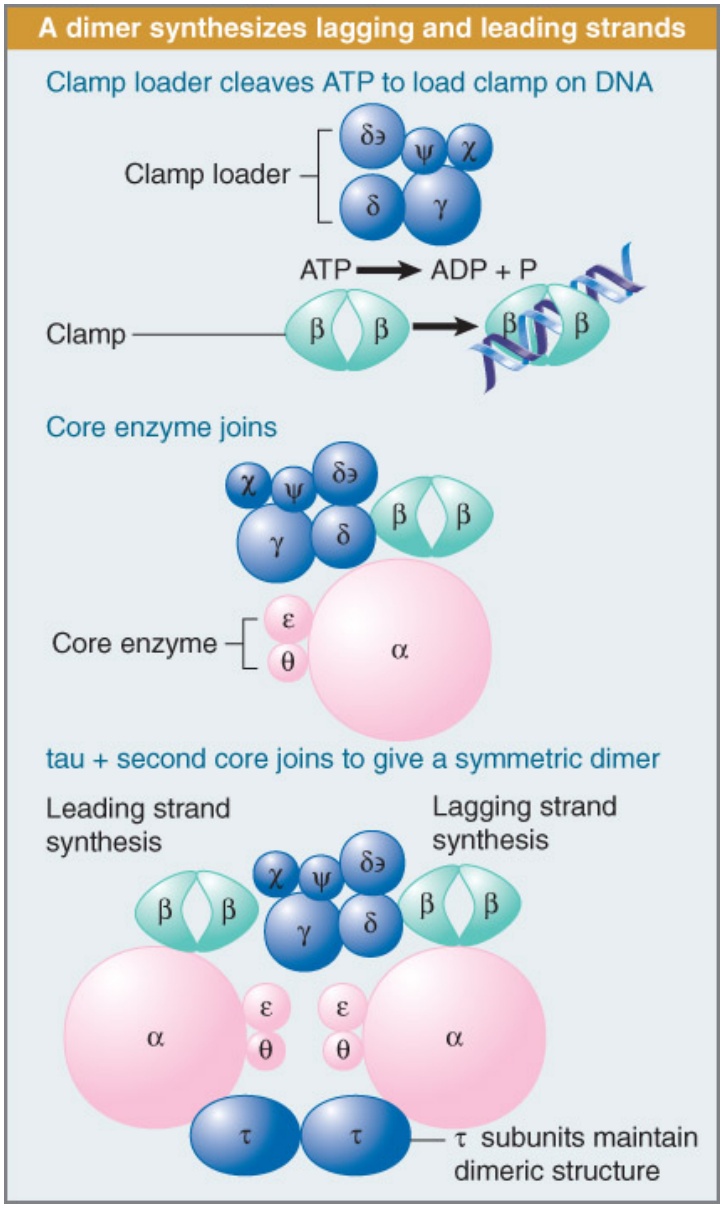



Each new DNA strand is synthesized by an individual catalytic unit. The behavior of these two units is different because the new DNA strands are growing in opposite directions. One enzyme unit is moving with the unwinding point and synthesizing the leading strand continuously. The other unit is moving "backwards," relative to the DNA, along the exposed single strand. Only short segments of template are exposed at any one time. When synthesis of one Okazaki fragment is completed, synthesis of the next Okazaki fragment is required to start at a new location approximately in the vicinity of the growing point for the leading strand. This requires a translocation relative to the DNA of the enzyme unit that is synthesizing the lagging strand.




The upper model for the action of lagging strand polymerase is that when an enzyme unit completes one Okazaki fragment, it moves to a new position to synthesize the next fragment. The lower model is that the lagging strand polymerase dissociates when it completes an Okazaki fragment, and a new enzyme unit associates with DNA to synthesize the next Okazaki fragment.

# DNA polymerase holoenzyme contains several subcomplexes




DNA polymerase III holoenzyme assembles in stages, generating an enzyme complex that synthesizes the DNA of both new strands.

·First the clamp loader uses hydrolysis of ATP to bind β subunits to a template-primer complex.

·Binding to DNA changes the conformation of the site on β that binds to the clamp loader, and as a result it now has a high affinity for the core polymerase. This enables core polymerase to bind, and this is the means by which the core polymerase is brought to DNA.

·A τ dimer binds to the core polymerase, and provides a dimerization function that binds a second core polymerase (associated with another β clamp). The holoenzyme is asymmetric, because it has only 1 clamp loader. The clamp loader is responsible for adding a pair of β dimers to each parental strand of DNA.

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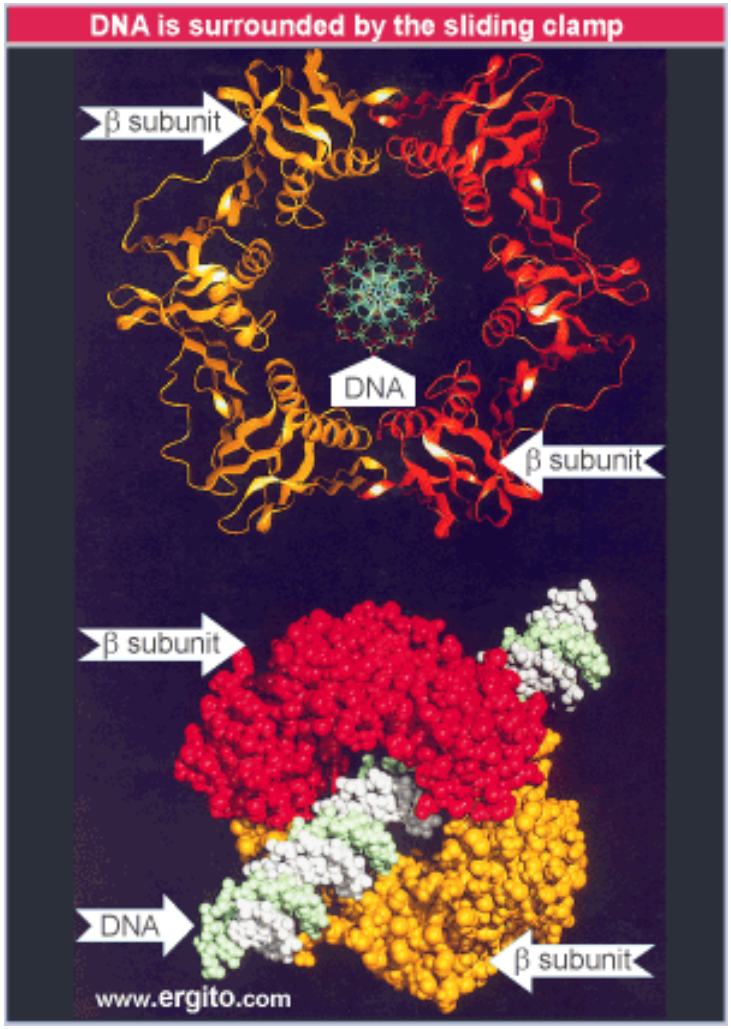
# β clamp




The β subunit of DNA polymerase III holoenzyme consists of a head to tail dimer (the two subunits are shown in red and orange) that forms a ring completely surrounding a DNA duplex (shown in the center). The β dimer makes the holoenzyme highly processive. β is strongly bound to DNA, but can slide along a duplex molecule. The ring has an external diameter of 80 Å and an internal cavity of 35 Å, almost twice the diameter of the DNA double helix (20 Å). The space between the protein ring and the DNA is filled by water. Each of the β subunits has three globular domains with similar organization (although their sequences are different). Thus, the dimer has 6-fold symmetry, reflected in 12 α-helices that line the inside of the ring. The dimer surrounds the duplex, providing the "**sliding clamp**" that allows the holoenzyme to slide along DNA. The structure explains the high processivity—there is no way for the enzyme to fall off!

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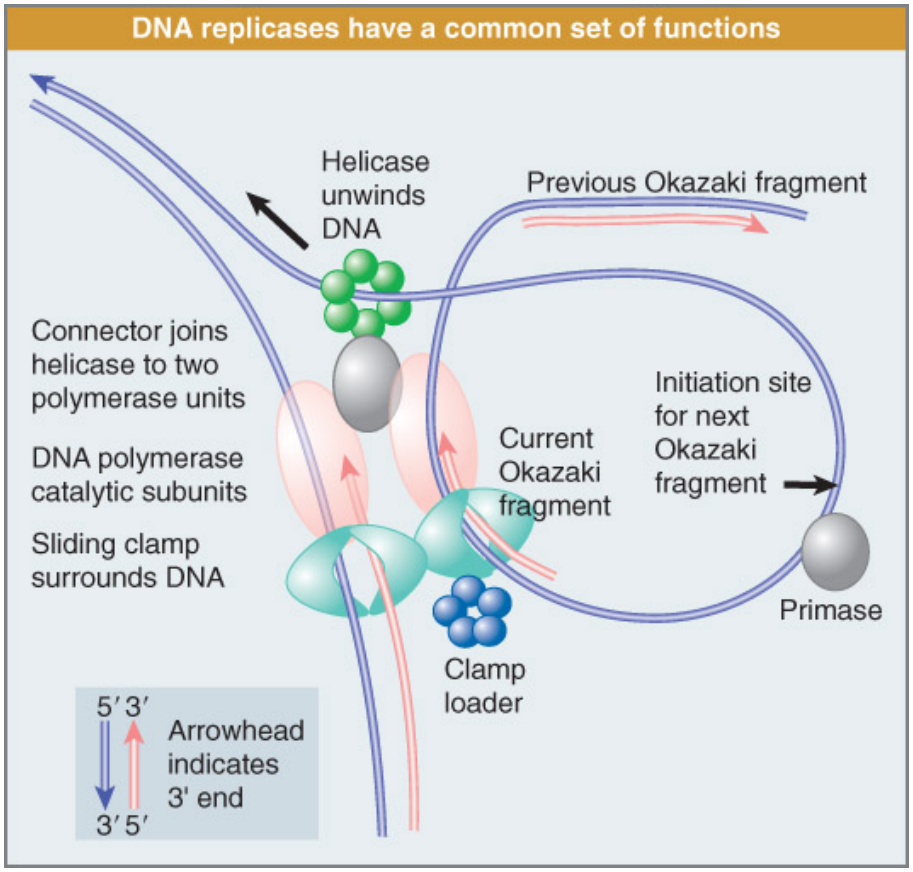
# The looping model




The helicase creating the replication fork is connected to two DNA polymerase catalytic subunits, each of which is held on to DNA by a sliding clamp. The polymerase that synthesizes the leading strand moves continuously. The polymerase that synthesizes the lagging strand dissociates at the end of an Okazaki fragment and then reassociates with a primer in the single-stranded template loop to synthesize the next fragment.

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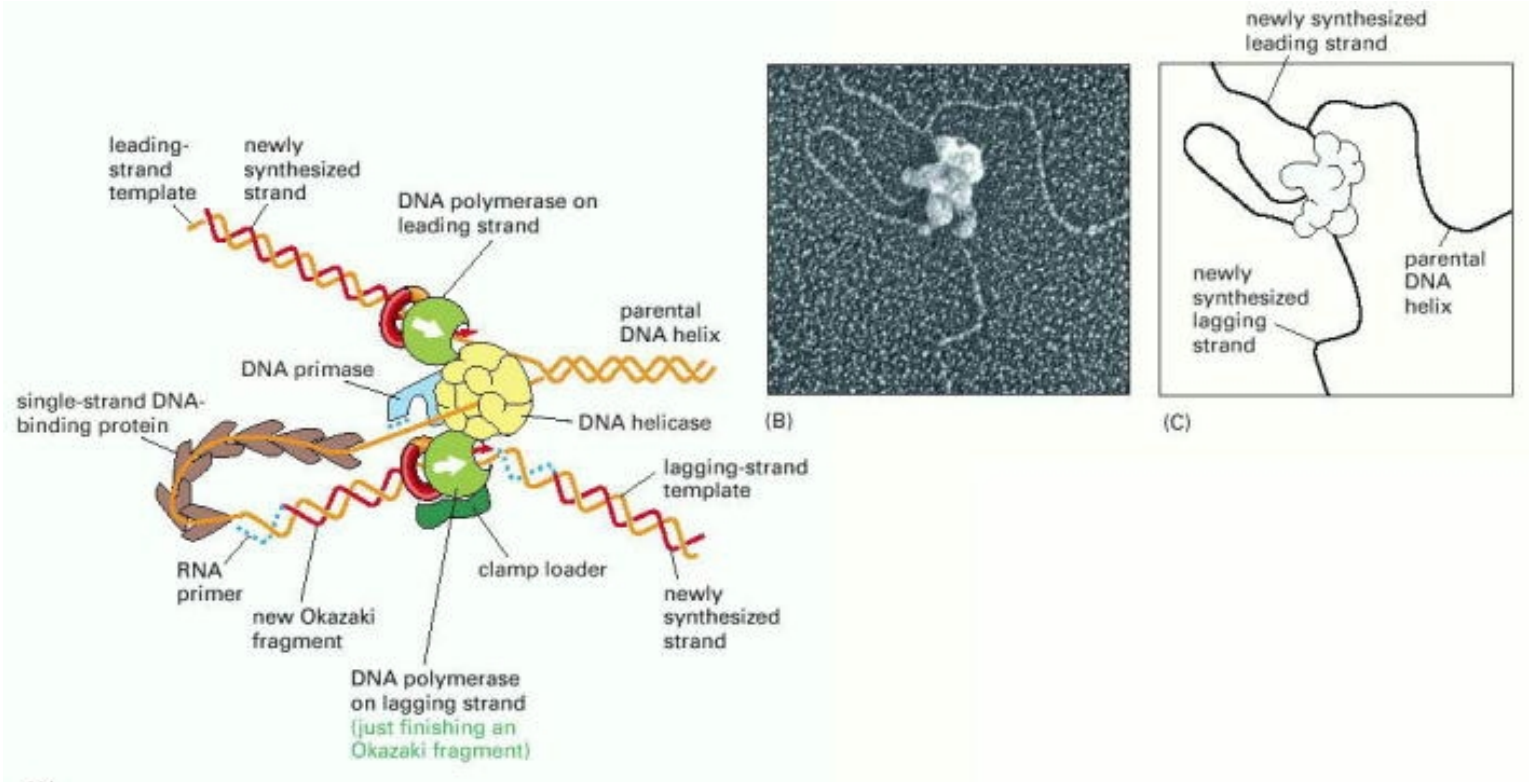
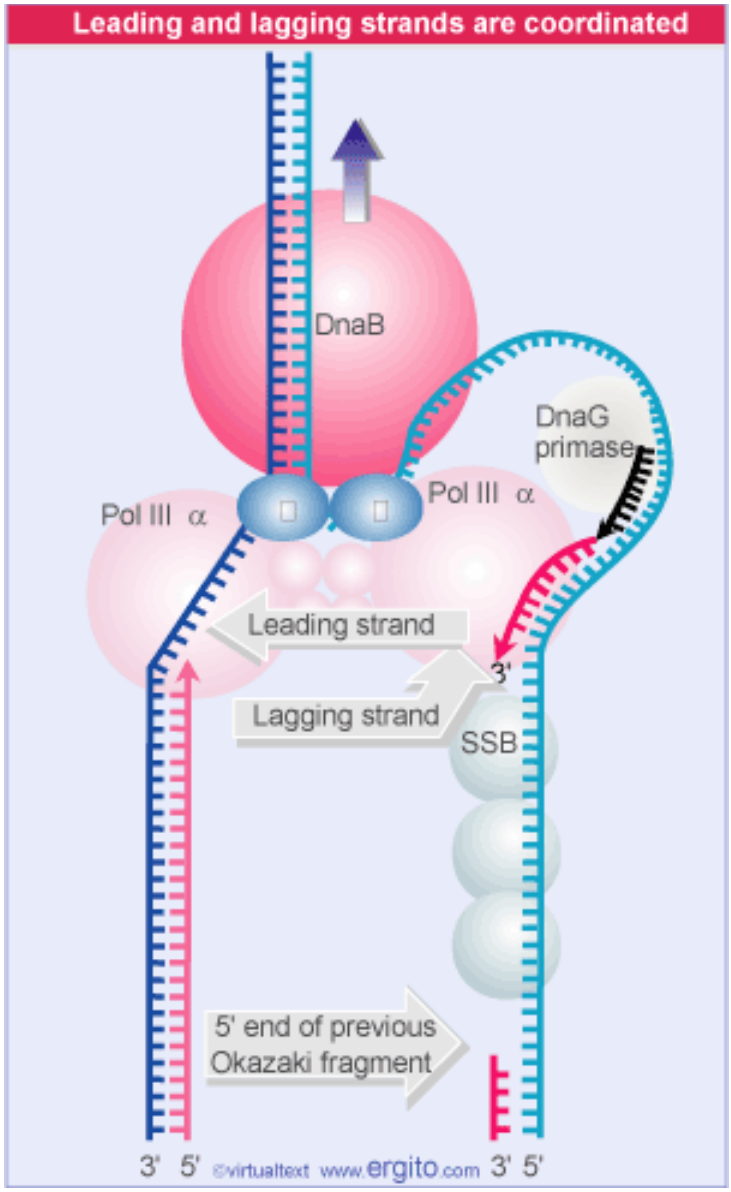
# The looping model evidence




(A) This schematic diagram shows a current view of the arrangement of replication at a replication fork when the fork is moving. The diagram has been altered by folding the DNA on the lagging strand to bring the lagging strand DNA polymerase molecule into a complex with the leading strand DNA polymerase molecule. This folding process also brings the 3' end of each completed Okazaki fragment close to the start site for the next fragment. Because the lagging strand DNA polymerase molecule remains bound to the rest of the replication proteins, it can be reused to synthesize successive Okazaki fragments. (B) An electron micrograph showing the replication machine from the bacteriophage T4 as it moves along a template synthesizing DNA behind it. The micrograph was from Jack Griffith. (C) An interpretation of the micrograph shown in B.




Each catalytic core of Pol III synthesizes a daughter strand. DnaB is responsible for forward movement at the replication fork.
The basic principle that is established by the dimeric polymerase model is that, while one polymerase subunit synthesizes the leading strand continuously, the other cyclically initiates and terminates the Okazaki fragments of the lagging strand within a large single-stranded loop formed by its template strand. The replication fork is created by a helicase, typically forming a hexameric ring, that translocates in the 5′—3′ direction on the template for the lagging strand. The helicase is connected to two DNA polymerase catalytic subunits, each of which is associated with a sliding clamp.

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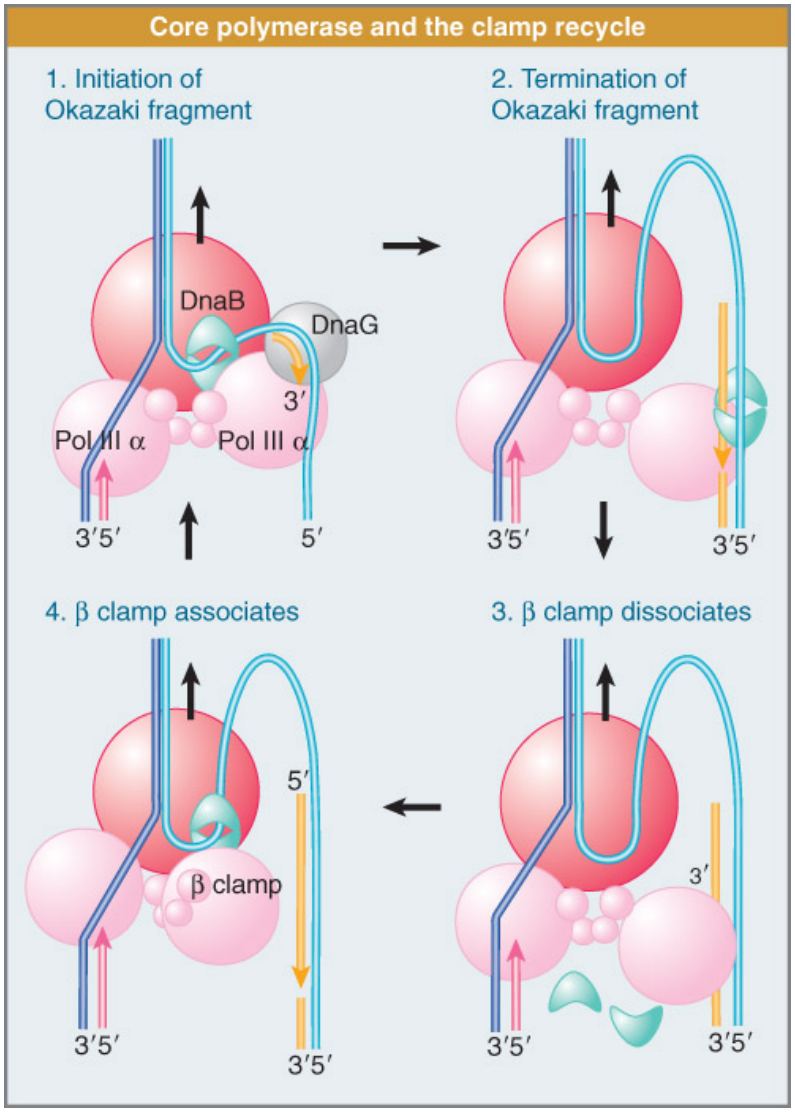


Core polymerase and the β clamp dissociate at completion of Okazaki fragment synthesis and reassociate at the beginning.

**What happens when the Okazaki fragment is completed?** All of the components of the replication apparatus function processively (that is, they remain associated with the DNA), except for the primase and the β clamp, which dissociate when the synthesis of each fragment is completed. A new β clamp is then recruited by the clamp loader to initiate the next Okazaki fragment. The lagging strand polymerase transfers from one β clamp to the next in each cycle, without dissociating from the replicating complex.

**What is responsible for recognizing the sites for initiating synthesis of Okazaki fragments?** In *oriC* replicons, the connection between priming and the replication fork is provided by the dual properties of DnaB: it is the helicase that propels the replication fork; and it interacts with the **DnaG primase** at an appropriate site. Following primer synthesis, the primase is released. The length of the priming RNA is limited to 8-14 bases. Apparently DNA polymerase III is responsible for displacing the primase.

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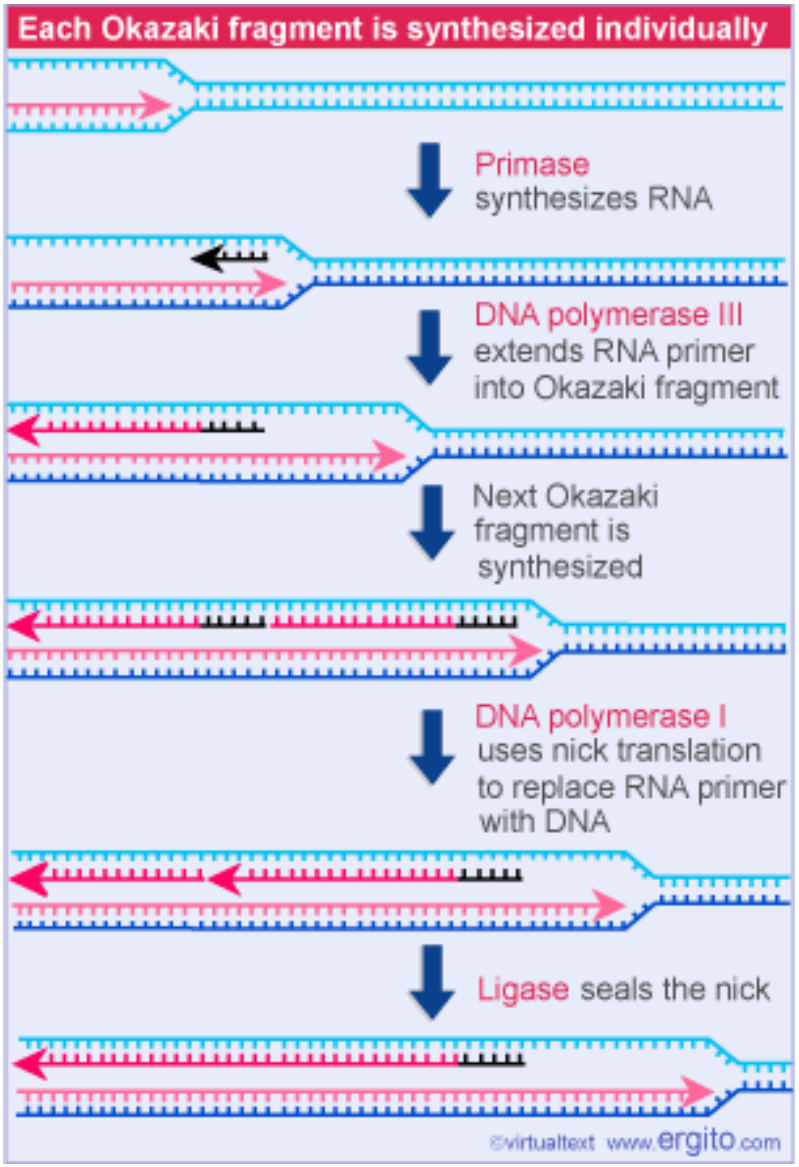
# Okazaki fragments are linked by ligase




Synthesis of Okazaki fragments requires priming, extension, removal of RNA, gap filling, and nick ligation.

**DNA polymerase I** removes the primer and replaces it with DNA in an action that resembles nick translation.

**DNA ligase** makes the bond that connects the 3′ end of one Okazaki fragment to the 5′ beginning of the next fragment.

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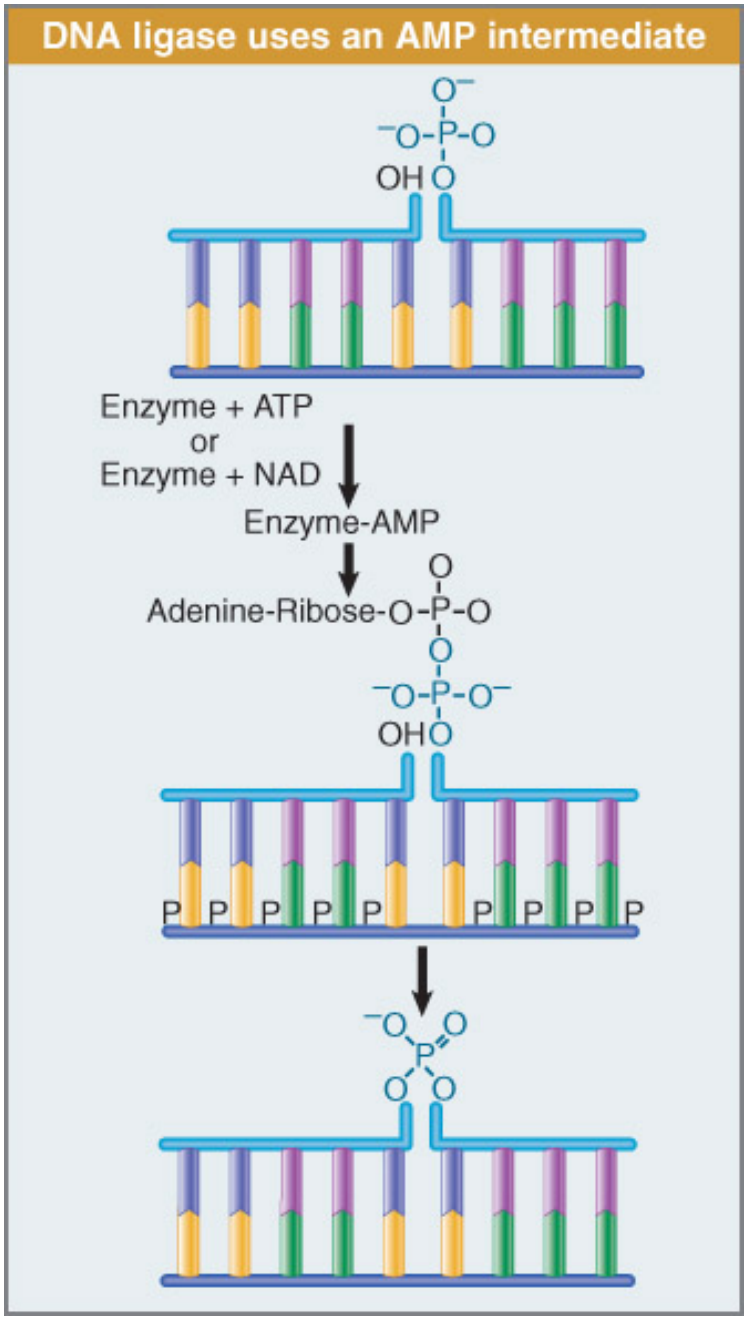


DNA ligase seals nicks between adjacent nucleotides by employing an enzyme-AMP intermediate.

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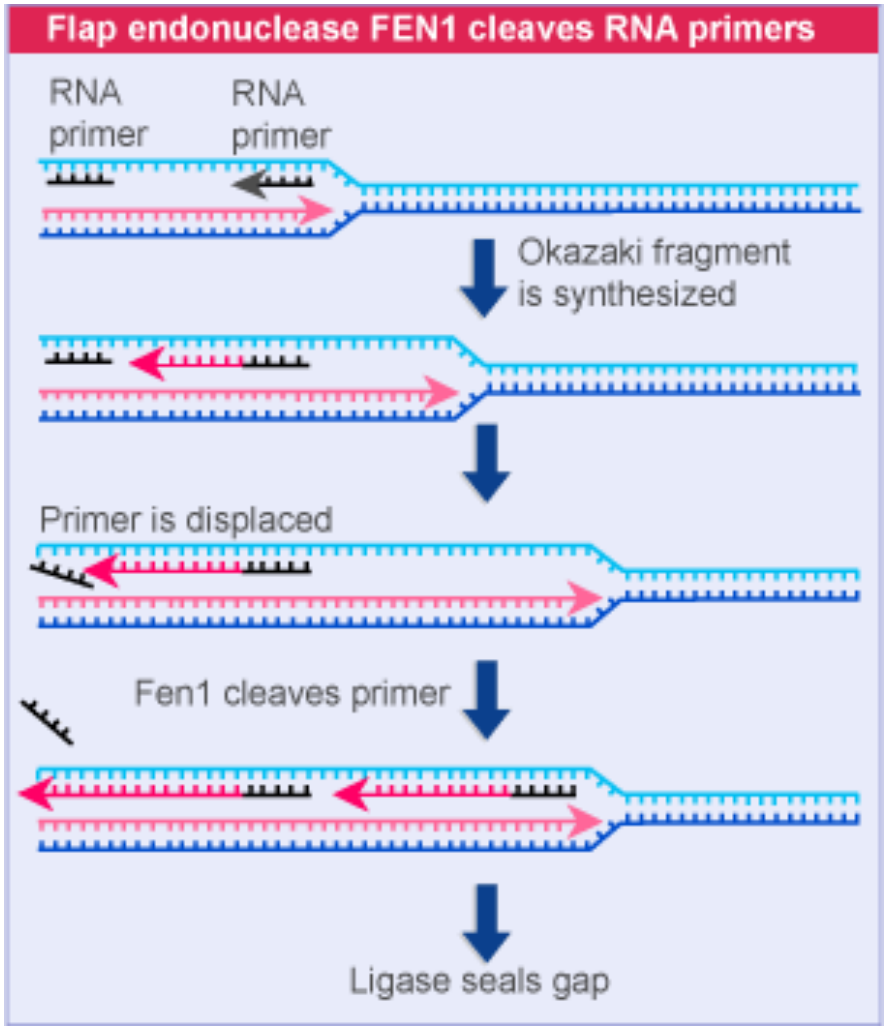


**FEN1 is an exo/endonuclease** that recognizes the structure created when one strand of DNA is displaced from a duplex as a "flap". In replication it cleaves at the base of the flap to remove the RNA primer.

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## Eukaryotic DNA polymerases

| DNA polymerases undertake replication or repair | | |
|---|---|---|
| DNA polymerase | Function | Structure |
| | High fidelity replicases | |
| α | Nuclear replication | 350 kD tetramer |
| δ | " | 250 kD tetramer |
| ε | " | 350 kD tetramer |
| γ | Mitochondrial replication | 200 kD dimer |
| | High fidelity repair | |
| β | Base excision repair | 39 kD monomer |
| | Low fidelity repair | |
| ζ | Thymine dimer bypass | heteromer |
| η | Base damage repair | monomer |
| ι | Required in meiosis | monomer |
| κ | Deletion and base substitution | monomer |



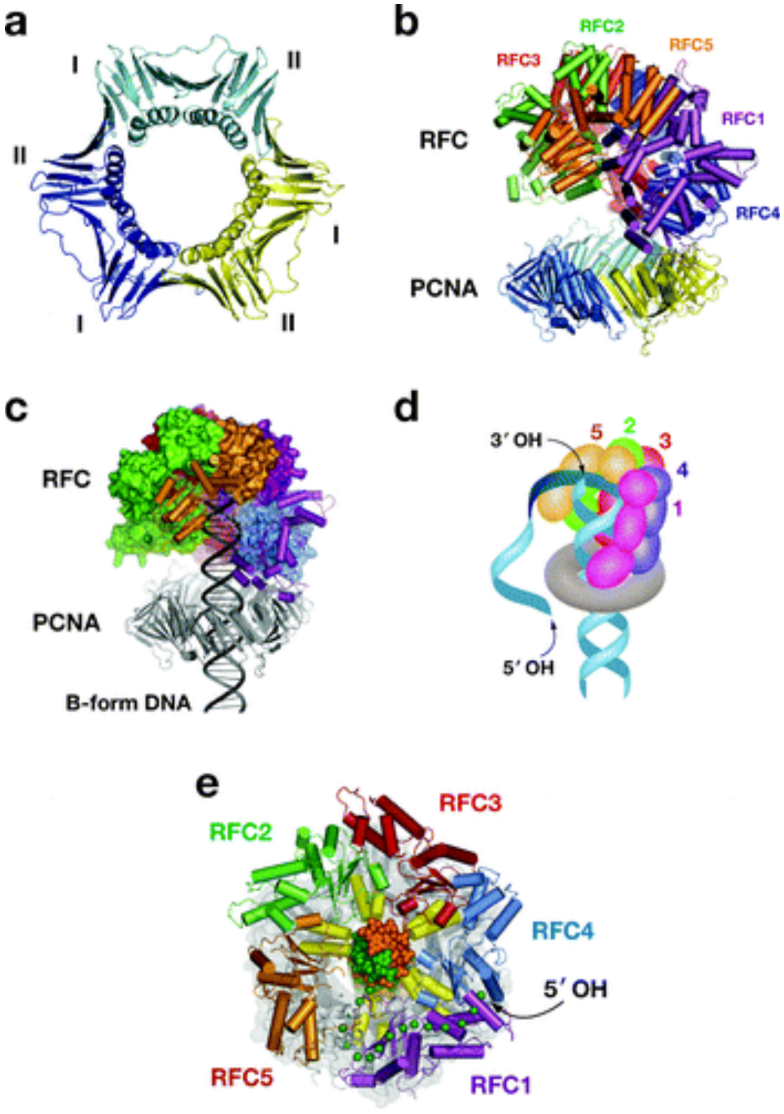
Eukaryotic replication fork contains 1 complex of DNA polymerase α/primase and 2 complexes of DNA polymerase δ and/or ε.

The DNA polymerase α/primase complex initiates the synthesis of both DNA strands.

DNA polymerase δ elongates the leading strand and a second DNA polymerase δ or DNA polymerase ε elongates the lagging strande lagging.

Eukaryotic cells have many DNA polymerases. The replicative enzymes operate with high fidelity. Except for the β enzyme, the repair enzymes all have low fidelity. Replicative enzymes have large structures, with separate subunits for different activities. Repair enzymes have much simpler structures.

# Eukaryotic clamp and clamp loader




Structures of the eukaryotic clamp and clamp loader from Saccharomyces cerevisiae. (a) View of the C-terminal face of PCNA. The ring-shaped PCNA is a head-to-tail trimer of a two-domain monomer. The sixfold pseudosymmetry of the β clamp is evident in PCNA as well. (b) The structure of replication factor C (RFC) bound to PCNA reveals the structural similarity between RFC and γ complex. RFC binds to the C-terminal face of PCNA. (c–e) The RFC subunits are arranged in a helix that tracks the minor groove of B-form DNA modeled through the PCNA ring. (d) In this cartoon, the 5' terminus of a recessed primer template is positioned to exit the central channel of the clamp and clamp loader through the gap between RFC1 and RFC5. (e) N-terminal regions of the five RFC subunits and the PCNA ring from the RFC-PCNA structure. Two conserved helices in each RFC subunit (yellow) are in position to interact with DNA (orange/green) that passes through the central channel of PCNA (gray) with the 5' terminus (green spheres) exiting between RFC1 and RFC5.

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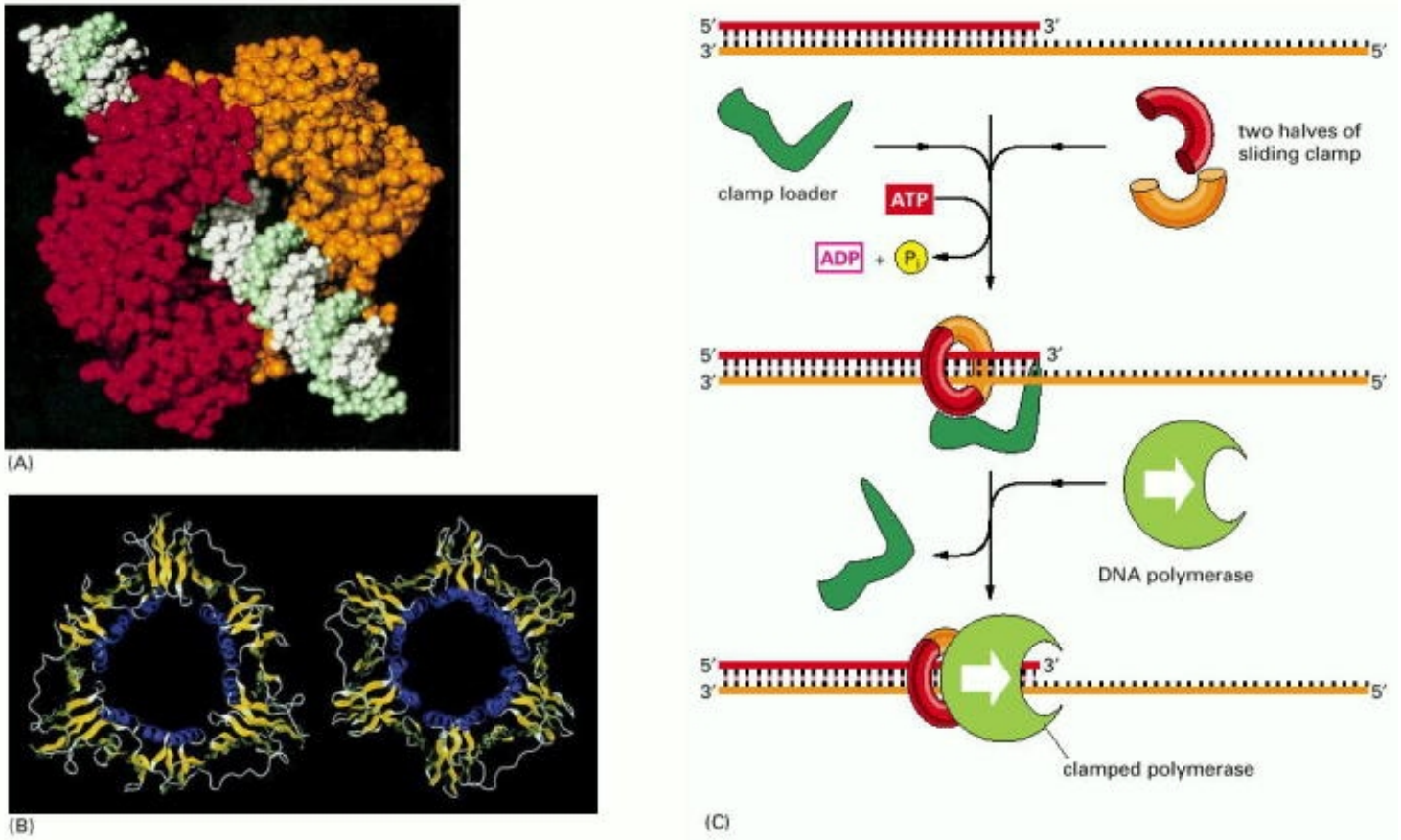
# The regulated sliding clamp that holds DNA polymerase on the DNA




(A) The structure of the clamp protein from *E. coli*, as determined by x-ray crystallography, with a DNA helix added to indicate how the protein fits around DNA. (B) The clamp protein in eucaryotes is called PCNA, a homotrimer. (C) Schematic illustration showing how the clamp is assembled to hold a moving DNA polymerase molecule on the DNA. The clamp loader dissociates into solution once the clamp has been assembled. At a true replication fork, the clamp loader remains close to the lagging-strand polymerase, ready to assemble a new clamp at the start of each new Okazaki fragment. (*Cell* 69:425–437, 1992)

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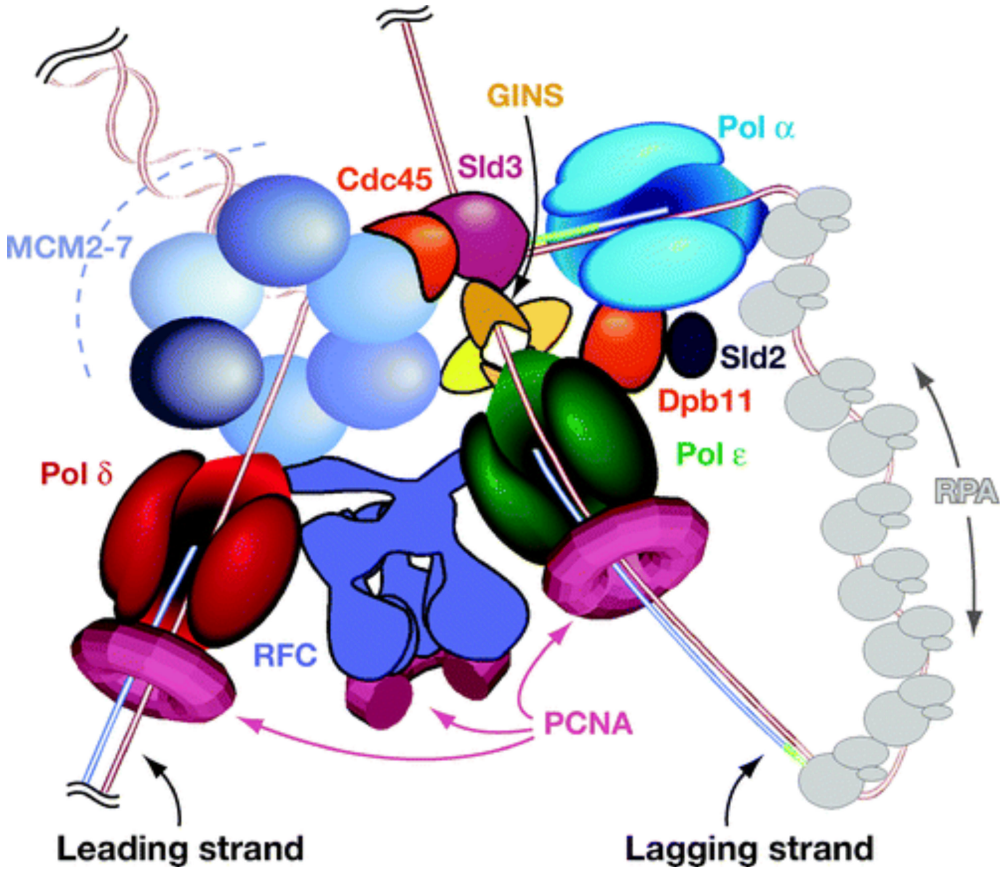
# Components Involved in DNA Replication

| Function | E. coli | Eukaryote |
|---|---|---|
| Helicase | DnaB | MCM complex |
| Loading helicase | DnaC | cdc6 |
| Single strand binding | SSB | RPA |
| Priming | DnaG | Polα/primase |
| Sliding clamp | β | PCNA |
| Clamp loading (ATPase) | γδ complex | RFC |
| Catalysis | Pol III core | Polδ + Polε |
| Holoenzyme dimerization | τ | ? |
| RNA removal | Pol I | FEN1 |
| Ligation | Ligase | Ligase 1 |

# Controversial issues in DNA replication: DNA polymease involvement in lagging and leading strands




Hypothetical arrangement of proteins at the eukaryotic replication fork. The hexameric MCM complex encircles the leading strand. In this cartoon, Pol δ is placed on the leading strand and Pol ε on the lagging, with RFC bridging the two polymerases and helicase. Pol α/primase action places it on the lagging strand along with RPA bound to the looping single-stranded DNA. Other factors involved in replication and known to bind certain proteins at the replication fork include Cdc45, Sld2, Sld3, Dpb11, and the heterotetrameric GINS complex.

O'Donnell, Annual Review of Biochemistry, Vol. 74: 283-315, 2005

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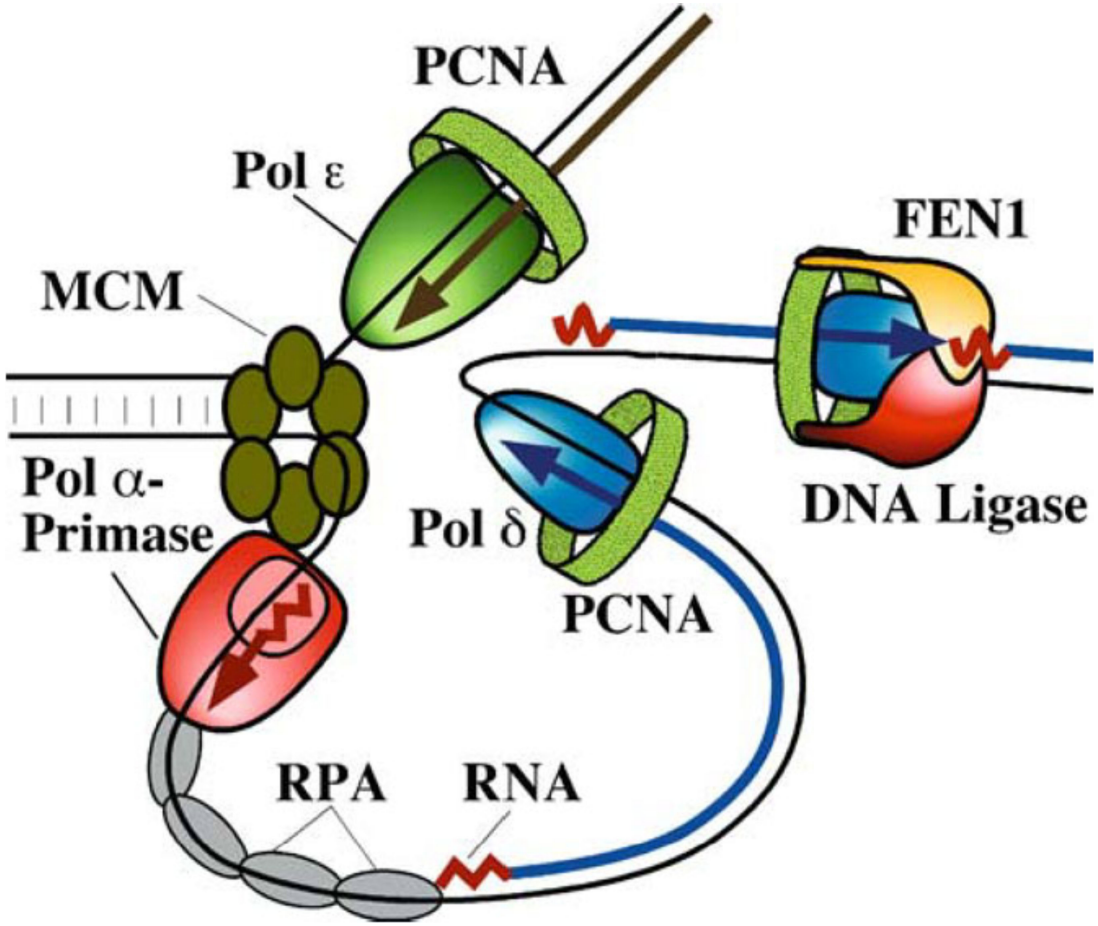
# Controversial issues in DNA replication: DNA polymease involvement in lagging and leading strands




Garg and Burgers, Crit. Rev. Bioch. Mol. Biol., 40:115-28, 2005

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# Telomeres and telomerase

**Elizabeth Blackburn**

**Carol Greider**

**Jack Szostak**

Dr. Elizabeth H. Blackburn, Professor at the University of California, San Francisco, discovered the molecular nature of telomeres - the ends of eukaryotic chromosomes that serve as protective caps essential for preserving the genetic information - and the ribonucleoprotein enzyme, telomerase, for which Dr. Blackburn was awarded awarded the 2009 **Nobel Prize** in Physiology or Medicine, together with Drs. Carol W. Greider (University of California, San Diego) and Jack W. Szostak (Harvard University).

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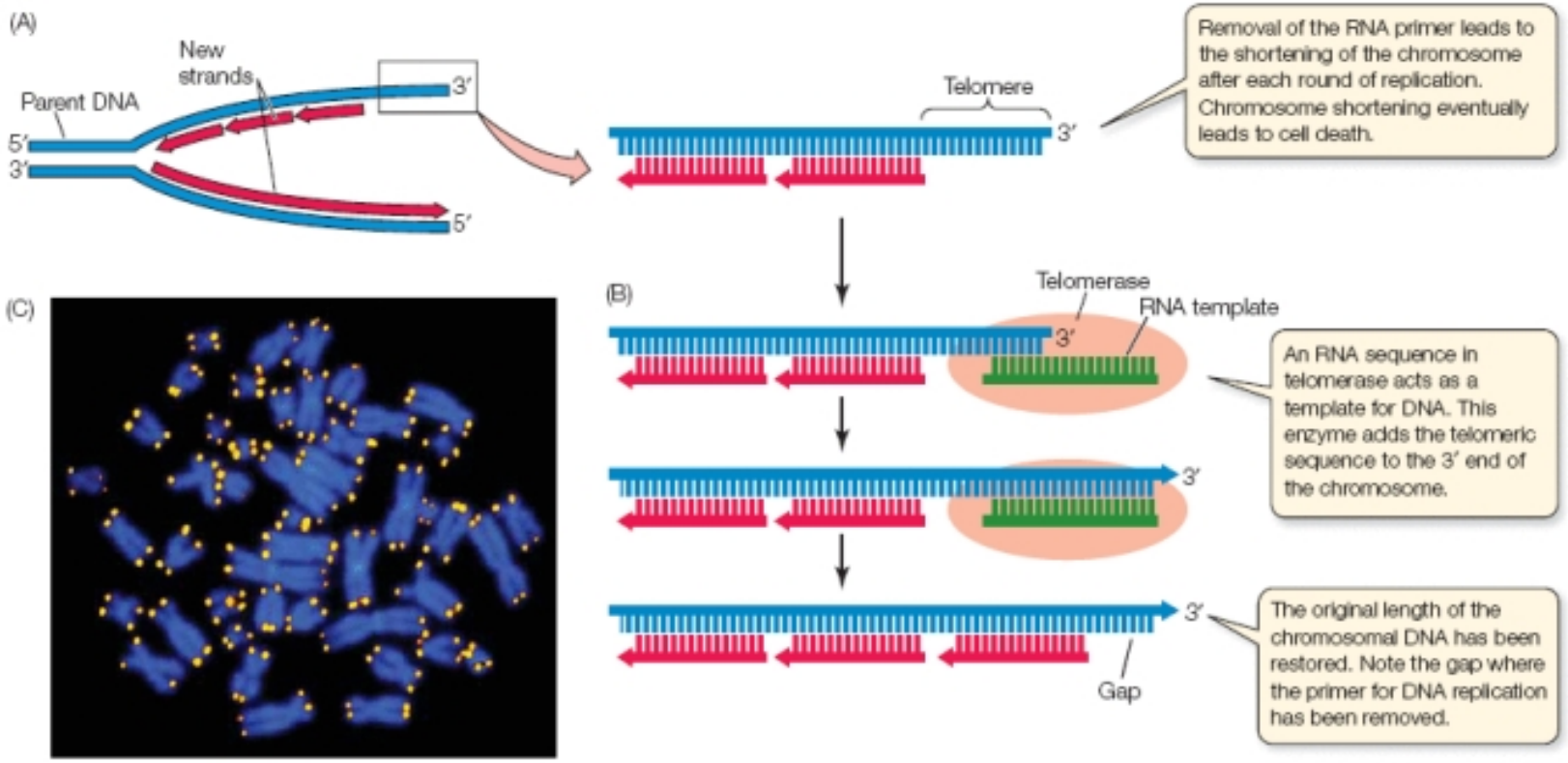
# Telomeres and telomerase




(A) Removal of the RNA primer at the 3' end of the lagging strand leaves a region of DNA--the telomere--unreplicated. (B) The enzyme telomerase binds to the 3' end and extends the lagging strand of DNA. An RNA sequence embedded in telomerase provides a template so that, overall, the DNA does not get shorter. (C) Bright fluorescent staining marks the telomeric regions on these blue-stained human chromosomes.

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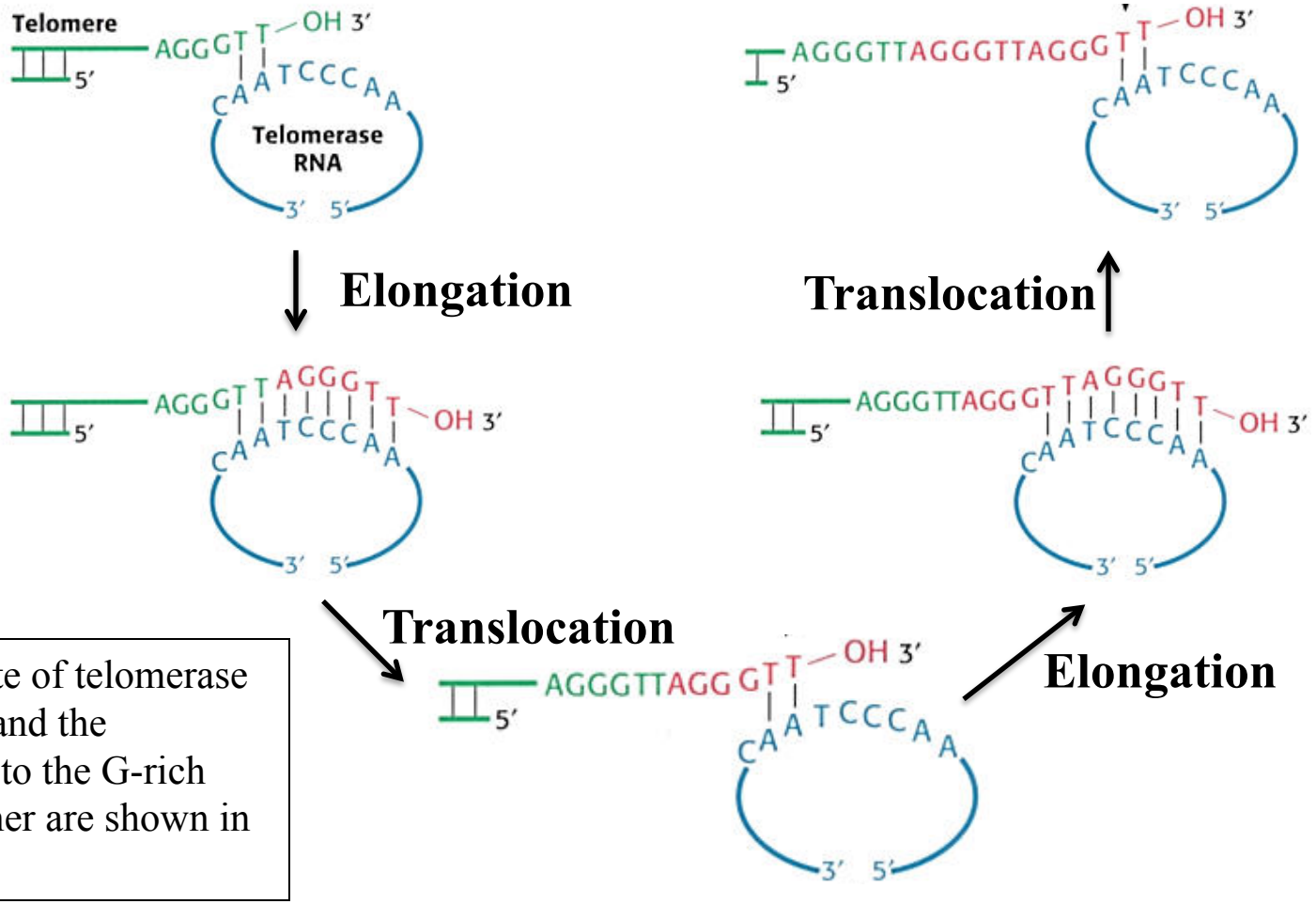
# Telomere synthesis




The RNA template of telomerase is shown in blue and the nucleotide added to the G-rich strand of the primer are shown in red.

Blackburn EH, Nature 350: 569-573, 1991

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# Accessary proteins in DNA replication

- RNA primase
- PCNA
- FRC
- Helicase
- Single strand DNA binding protein (SSB in E. coli and RPA in eukaryotes)
- Ligase

# Creating the replication forks at an origin


The minimal origin is defined by the distance between the outside members of the 13-mer and 9-mer repeats.
Initiation of replication at *oriC in vitro* starts with formation of a complex that requires six proteins: **DnaA, DnaB, DnaC, HU, Gyrase, and SSB**.

6 DnaC monomers bind each hexamer of DnaB and this complex binds to the origin.

A hexamer of DnaB forms the replication fork. Gyrase and SSB are also required.

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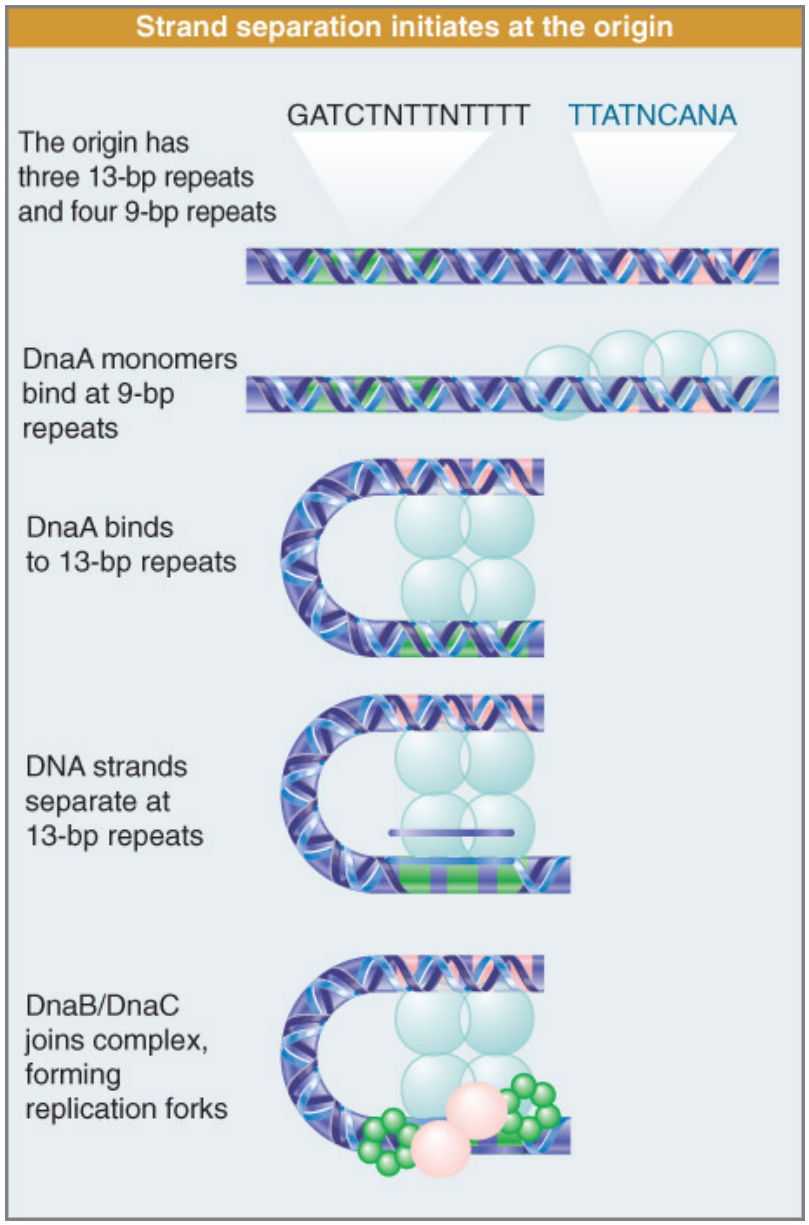
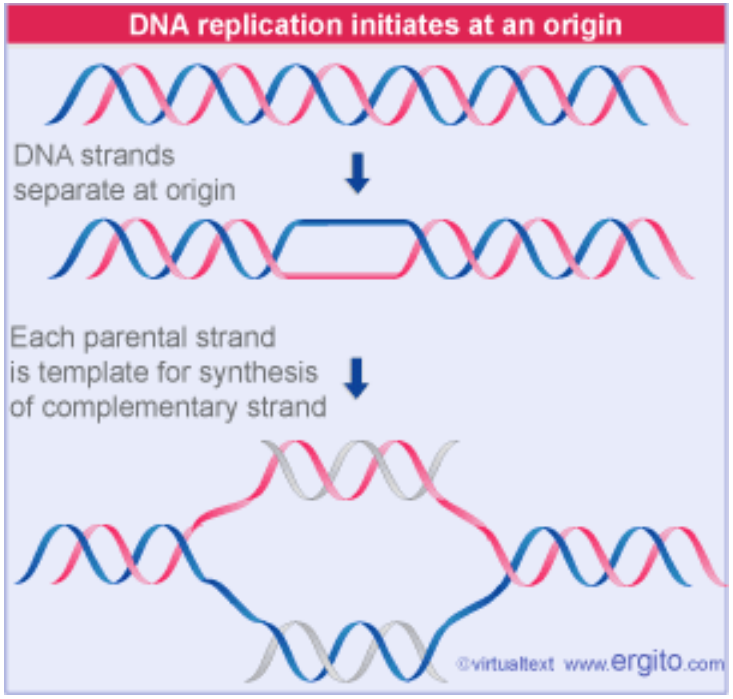
# DNA sequences of replication origin

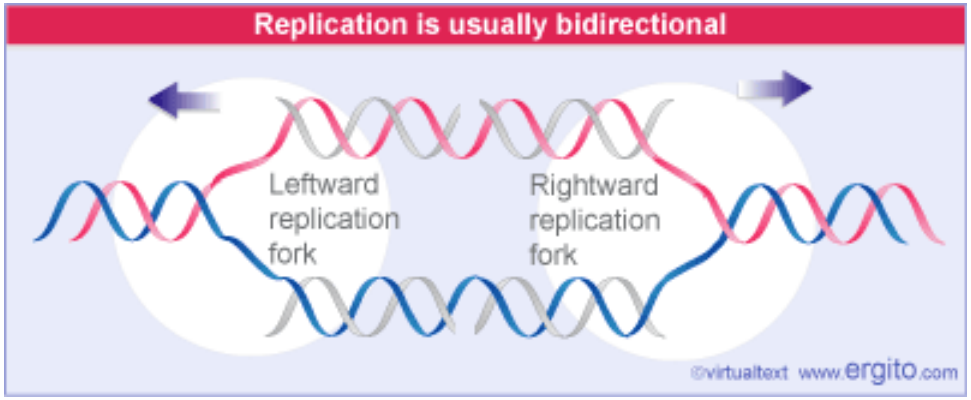



DnaA binds to short repeated sequences and forms an oligomeric complex that melts DNA. The four 9 bp consensus sequences on the right side of *oriC* provide the initial binding sites for DnaA. It binds cooperatively to form a central core around which *oriC* DNA is wrapped. Then DnaA acts at three A-T-rich 13 bp tandem repeats located in the left side of *oriC*. In the presence of ATP, DnaA melts the DNA strands at each of these sites to form an open complex. All three 13 bp repeats must be opened for the reaction to proceed to the next stage.

Altogether, 2-4 monomers of DnaA bind at the origin, and they recruit 2 "prepriming" complexes of DnaB-DnaC to bind, so that there is one for each of the two (bidirectional) replication forks. Each DnaB-DnaC complex consists of 6 DnaC monomers bound to a hexamer of DnaB. Each DnaB·DnaC complex transfers a hexamer of DnaB to an opposite strand of DNA. DnaC hydrolyzes ATP in order to release DnaB.

# Bidirectional replication

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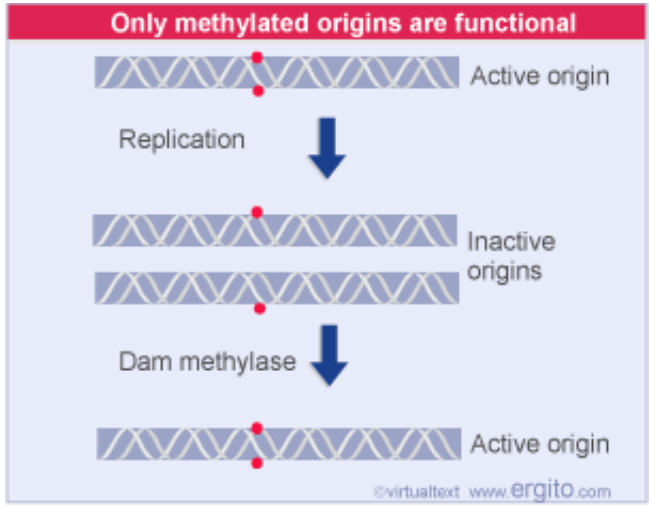
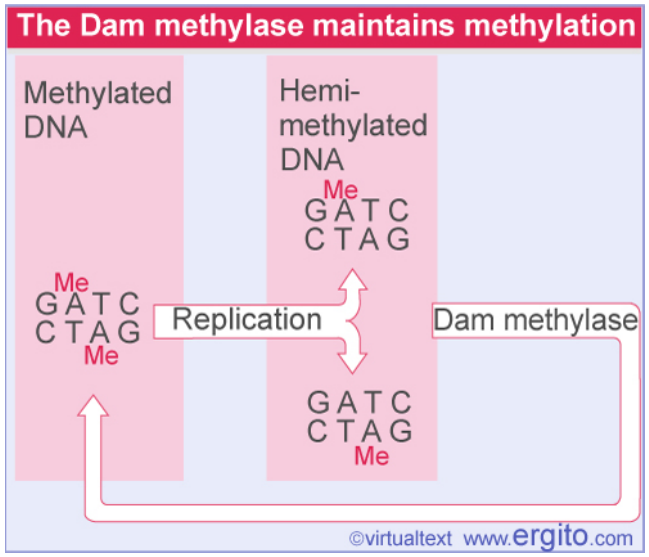
# Regulation of replication initiation




- Replication of methylated DNA gives hemimethylated DNA, which maintains its state at GATC sites until the Dam methylase restores the fully methylated condition.

- *oriC* contains 11 repeats that are methylated on adenine on both strands.

- Replication generates hemimethylated DNA, which cannot initiate replication.

- There is a 13 min delay before the repeats are remethylated.

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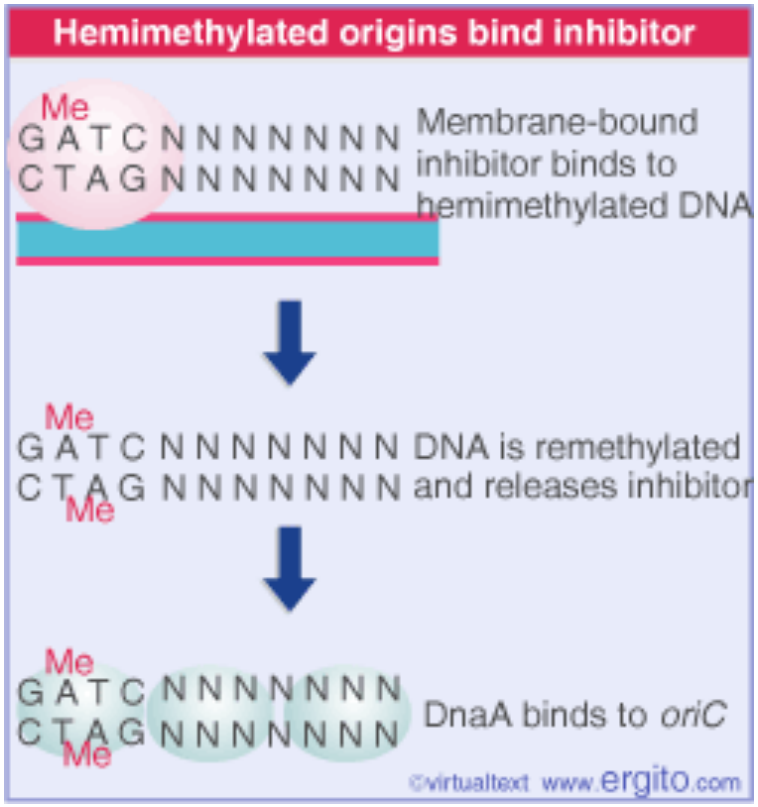
# Origins may be sequestered after replication




A membrane-bound inhibitor binds to hemimethylated DNA at the origin, and may function by preventing the binding of DnaA. It is released when the DNA is remethylated.

- SeqA binds to hemimethylated DNA and is required for delaying rereplication.
- SeqA may interact with DnaA.
- While the origins are hemimethylated, they bind to the cell membrane, and may be unavailable to methylases.

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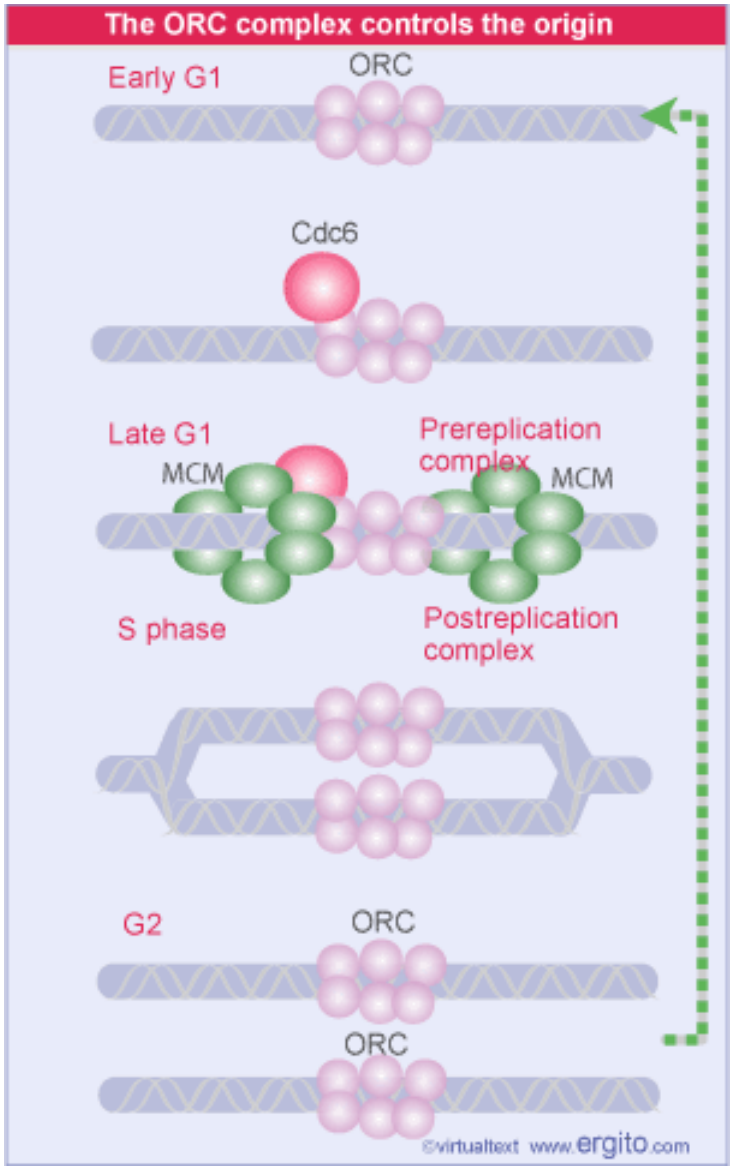


- Licensing factor is necessary for initiation of replication at each origin.

- Licensing factor is present in the nucleus prior to replication, but is inactivated or destroyed by replication. Initiation of another replication cycle becomes possible only after licensing factor reenters the nucleus after mitosis.

- The ORC proteins are associated with yeast origins throughout the cell cycle.

- Cdc6 protein is an unstable protein that is synthesized only in G1.

- Cdc6 binds to ORC and allows MCM proteins to bind. When replication is initiated, Cdc6 and MCM proteins are displaced. The degradation of Cdc6 prevents reinitiation.

- Some MCM proteins are in the nucleus throughout the cycle, but others may enter only after mitosis.

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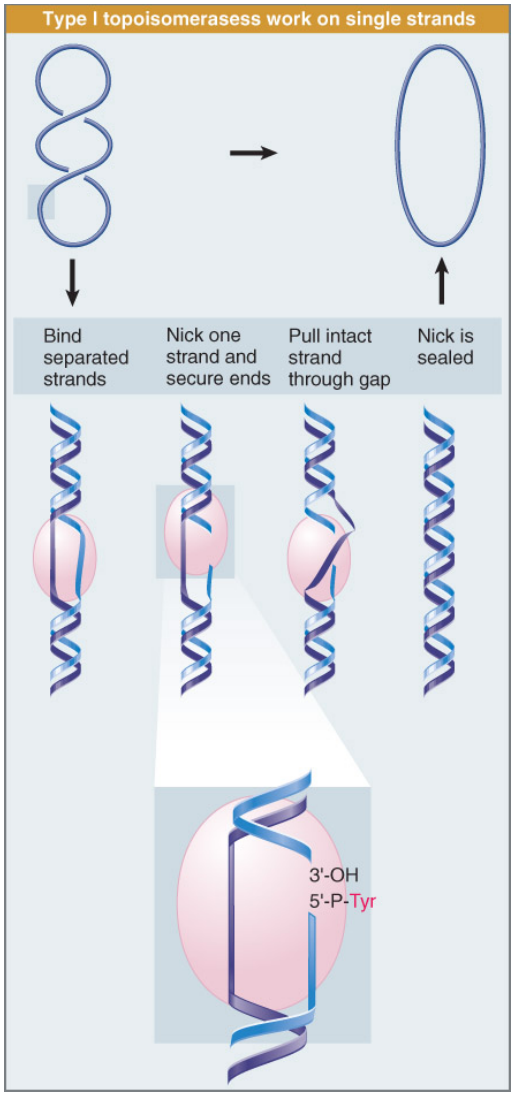
## Topoisomerases Relax or Introduce Supercoils in DNA

- Topoisomerases change the linking number by:
  - breaking bonds in DNA
  - changing the conformation of the double helix in space
  - remaking the bonds

- Type I enzymes act by breaking a single strand of DNA.

- Type II enzymes act by making double-strand breaks.

# Topoisomerases Break and Reseal Strands




- Type I topoisomerases function by:
  - forming a covalent bond to one of the broken ends
  - moving one strand around the other
  - transferring the bound end to the other broken end
- Bonds are conserved, and as a result no input of energy is required.

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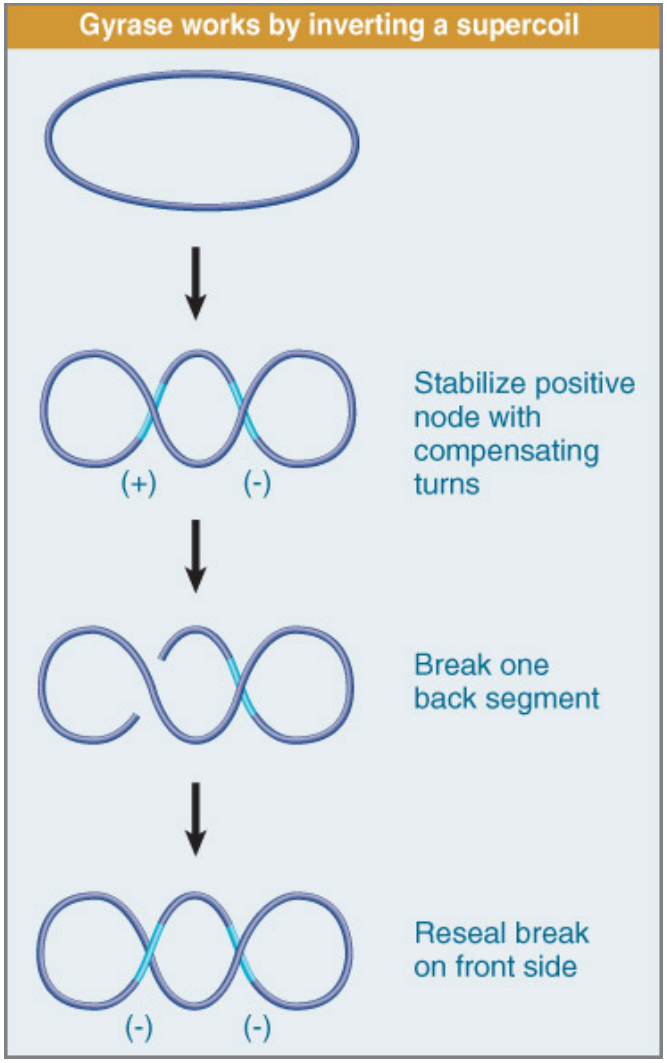
# Gyrase Functions by Coil Inversion




- *E. coli* gyrase is a type II topoisomerase.
  - It uses hydrolysis of ATP to provide energy to introduce negative supercoils into DNA.

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# Summary

• DNA synthesis occurs by semidiscontinuous replication, in which the leading strand of DNA growing 5′—3′ is extended continuously, but the lagging strand that grows overall in the opposite 3′—5′ direction is made as short Okazaki fragments, each synthesized 5′—3′. The leading strand and each Okazaki fragment of the lagging strand initiate with an RNA primer that is extended by DNA polymerase.

• The replisome contains an asymmetric dimer of DNA polymerase III; each new DNA strand is synthesized by a different core complex containing a catalytic (α) subunit. Processivity of the core complex is maintained by the β clamp, which forms a ring round DNA. The clamp is loaded on to DNA by the clamp loader complex. Clamp/clamp loader pairs with similar structural features are widely found in both prokaryotic and eukaryotic replication systems.

• The looping model for the replication fork proposes that, as one half of the dimer advances to synthesize the leading strand, the other half of the dimer pulls DNA through as a single loop that provides the template for the lagging strand. The transition from completion of one Okazaki fragment to the start of the next requires the lagging strand catalytic subunit to dissociate from DNA and then to reattach to a β clamp at the priming site for the next Okazaki fragment.

• DnaB provides the helicase activity at a replication fork; this depends on ATP cleavage. DnaB may function by itself in *oriC* replicons to provide primosome activity by interacting periodically with DnaG, which provides the primase that synthesizes RNA.

# Summary Cont'd

• Replication is initiated at *oriC* in *E. coli* when DnaA binds to a series of 9 bp repeats. This is followed by binding to a series of 13 bp repeats, where it uses hydrolysis of ATP to generate the energy to separate the DNA strands. The pre-priming complex of DnaC-DnaB displaces DnaA. DnaC is released in a reaction that depends on ATP hydrolysis; DnaB is joined by the replicase enzyme, and replication is initiated by two forks that set out in opposite directions.

•The availability of DnaA at the origin is an important component of the system that determines when replication cycles should initiate. Following initiation of replication, DnaA hydrolyzes its ATP under the stimulus of the β sliding clamp, generating an inactive form of the protein. Also, *oriC* must compete with the *dat* site for binding DnaA.

• Several sites that are methylated by the Dam methylase are present in the *E. coli* origin, including those of the 13-mer binding sites for DnaA. The origin remains hemimethylated and is in a sequestered state for ~10 minutes following initiation of a replication cycle. During this period it is associated with the membrane, and reinitiation of replication is repressed. The protein SeqA is involved in sequestration and may interact with DnaA.

• After cell division, nuclei of eukaryotic cells have a licensing factor that is needed to initiate replication. Its destruction after initiation of replication prevents further replication cycles from occurring in yeast. Licensing factor cannot be imported into the nucleus from the cytoplasm, and can be replaced only when the nuclear membrane breaks down during mitosis.

• The origin is recognized by the ORC proteins, which in yeast remain bound throughout the cell cycle. The protein Cdc6 is available only at S phase. In yeast it is synthesized during S phase and rapidly degraded. In animal cells it is synthesized continuously, but is exported from the nucleus during S phase. The presence of Cdc6 allows the MCM proteins to bind to the origin. The MCM proteins are required for initiation. The action of Cdc6 and the MCM proteins provides the licensing function.

• Telomeres are replicated by telomerase, a specialized polymerase that carries its own RNA template.

# DNA Recombination

## Definition

DNA rearrangements between precisely corresponding sequences

## Classification

1. General recombination (or homologous recombination)
2. Site-specific recombination

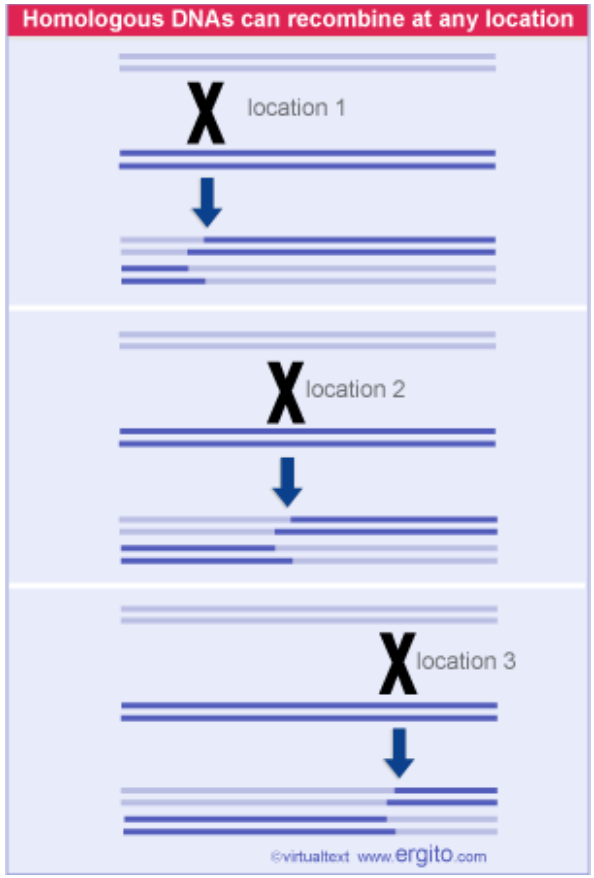
# Key Terms of Recombination

**Homologous recombination(generalized recombination)** involves a reciprocal exchange of sequences of DNA, e.g. between two chromosomes that carry the same genetic loci.

**Site-specific recombination(Specialized recombination)** occurs between two specific (not necessarily homologous) sequences, as in phage integration/ excision or resolution of cointegrate structures during transposition.

**Transposition** refers to the movement of a transposon to a new site in the genome.

# Homologous Recombination




**Key Concepts**

- Occurs at the “four strand” stage of meiosis during spermatogenesis or oogenesis
- Can occur anywhere in the homologous nucleotide sequences of the two participating strands
- Creates a heteroduplex region that can be thousands of base pairs long
- No nucleotide sequences altered at the site of exchange

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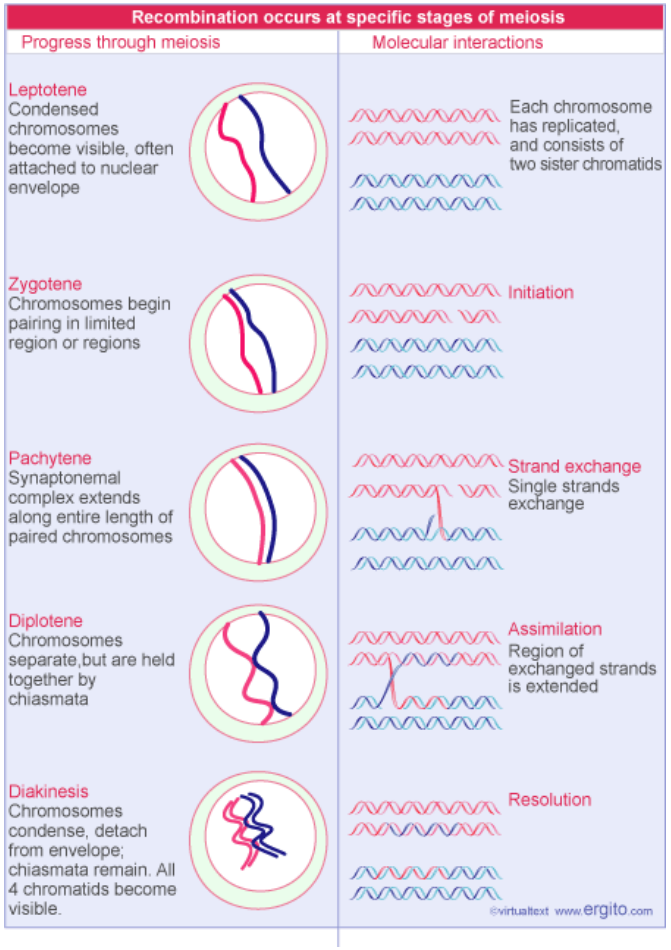
# Recombination Occurs at the Prophase of Meiosis




Recombination occurs during the first meiotic prophase of meiosis. This Figure compares the visible progress of chromosomes through the five stages of meiotic prophase with the molecular interactions that are involved in exchanging material between two of the four duplex DNAs.

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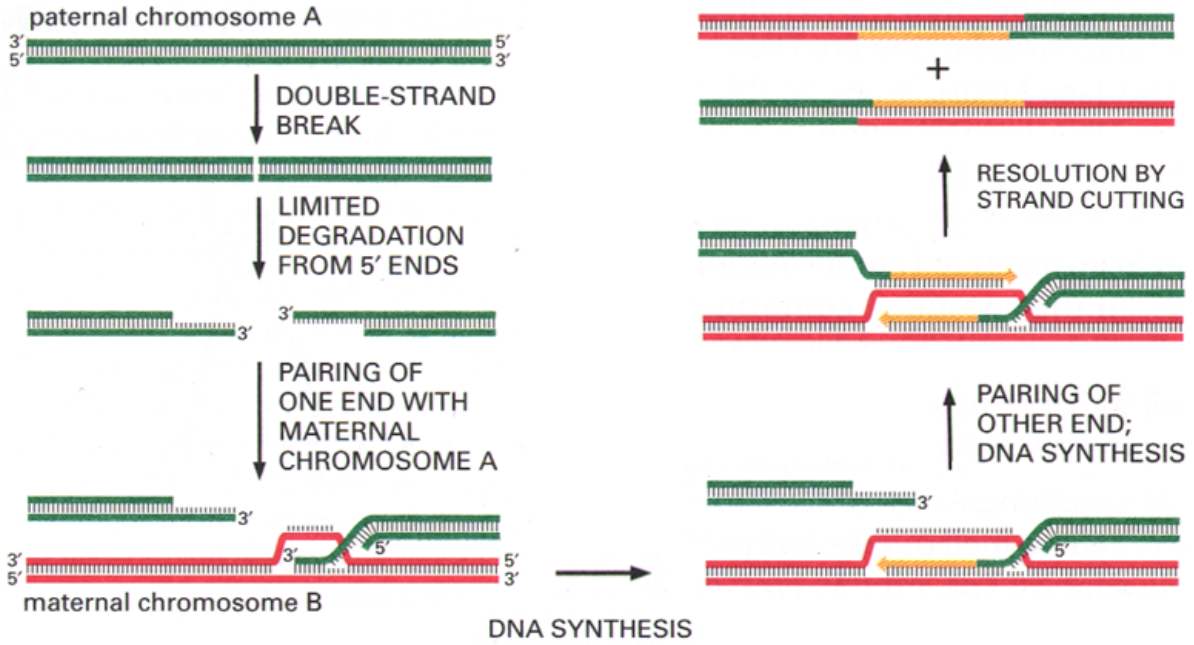
# Recombination in Meiosis




**Key Concepts**

- Recombination is initiated by making a double-strand break in one (recipient) DNA duplex.
- Exonuclease action generates 3’ single-stranded ends that invade the other (donor) duplex.
- New DNA synthesis replaces the material that has been degraded.
- This generates a recombinant joint molecule in which the two DNA duplexes are connected by heteroduplex DNA.

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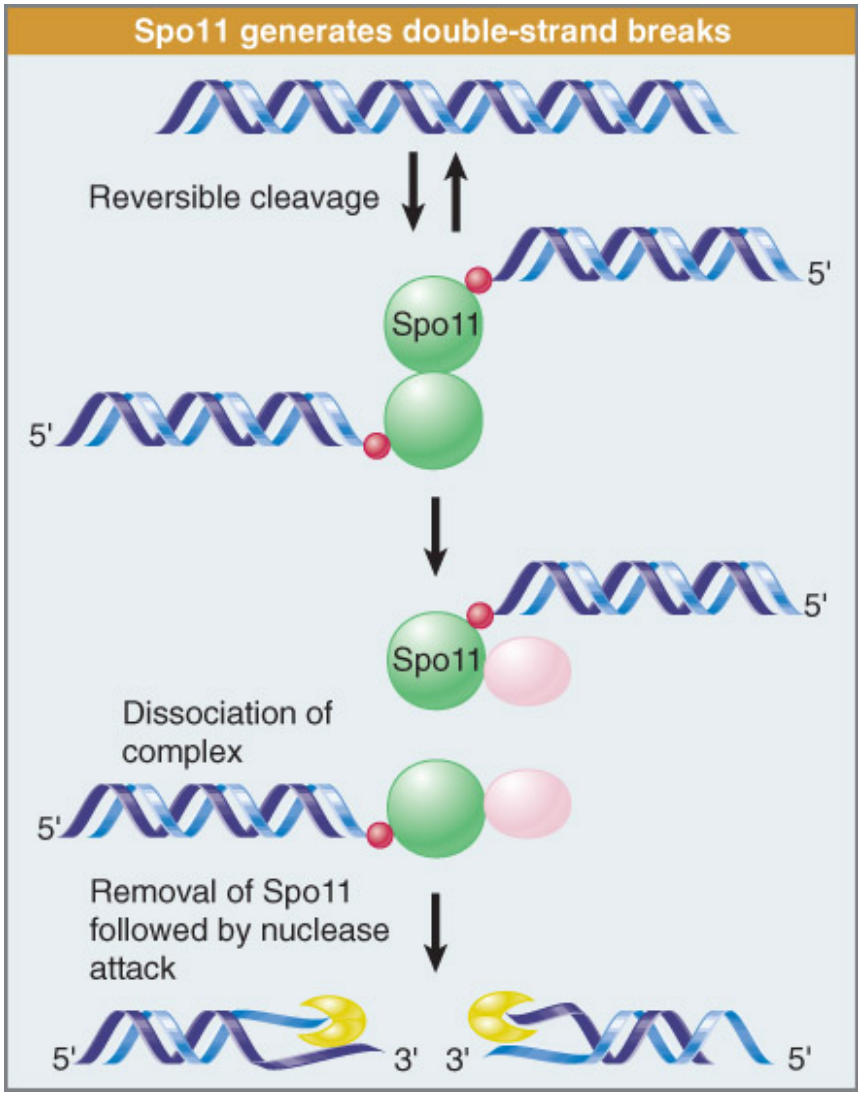
# Spo11 Creates DSBs




**Key Concepts**

•Double-strand breaks that initiate recombination occur before the synaptonemal complex forms.

•If recombination is blocked, the synaptonemal complex cannot form.

•Spo11 is homologous to the catalytic subunits of a family of type II topoisomerases.

•Spo11 interacts reversibly with DNA; the break is converted into a permanent structure by an interaction with another protein that dissociates the Spo11 complex.

•Removal of Spo11 is followed by nuclease action.

•At least 9 other proteins are required to process the double-strand breaks. One group of proteins is required to convert the double-strand breaks into protruding 3'–OH single-stranded ends. Another group then enables the single-stranded ends to invade homologous duplex DNA.

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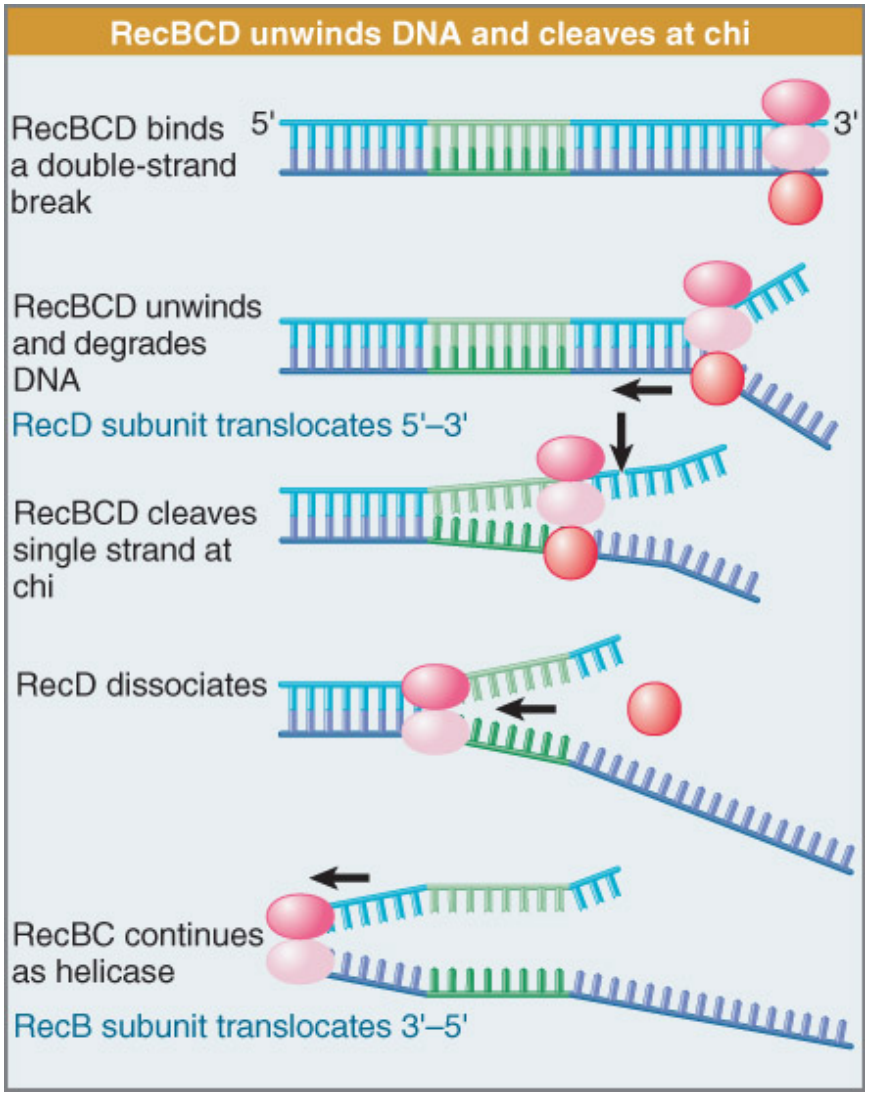
# Role of RecBCD in Recombination




## Key Concepts

•The RecBCD complex has nuclease and helicases activities.

•It binds to DNA downstream of a *chi* sequence, unwinds the duplex, and degrades one strand from 3'–5' as it moves to the *chi* site.

•The *chi* site triggers loss of the RecD subunit and nuclease activity.

## Chi Sequence

5' GCTGGTGG 3'
3' CGACCACC 5'

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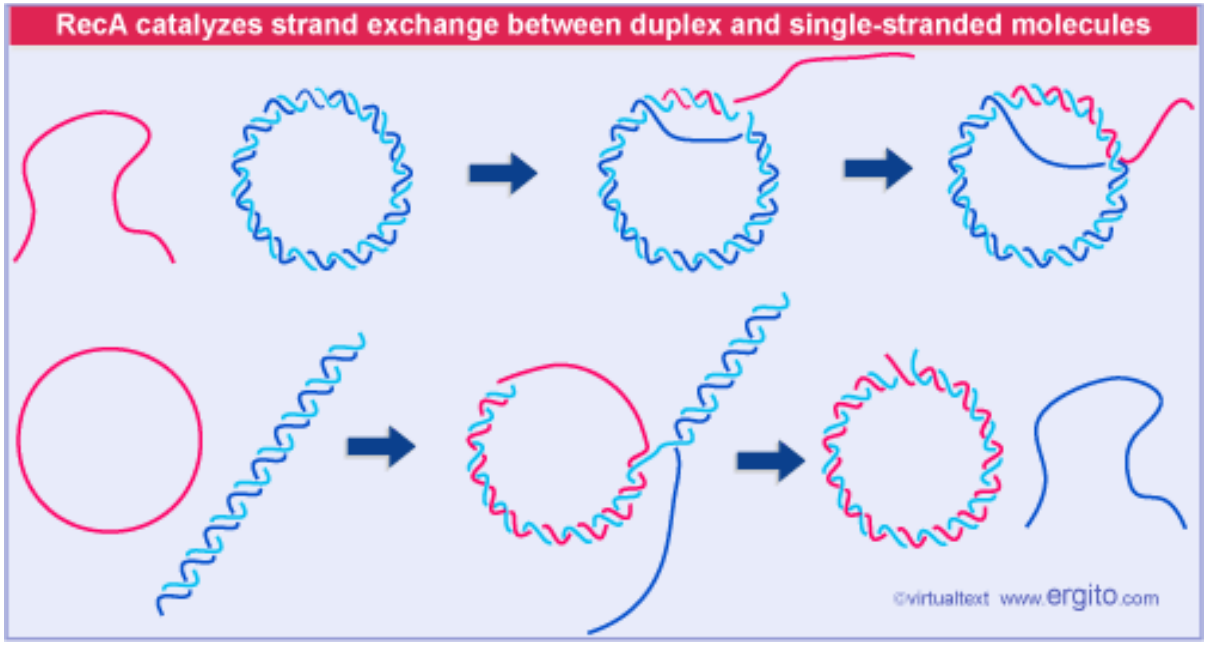
# RecA catalyzes Strand Exchange




## Key Concepts

RecA forms filaments with single-stranded or duplex DNA and catalyzes the ability of a single-stranded DNA with a free 3’ to displace its counterpart in a DNA duplex.

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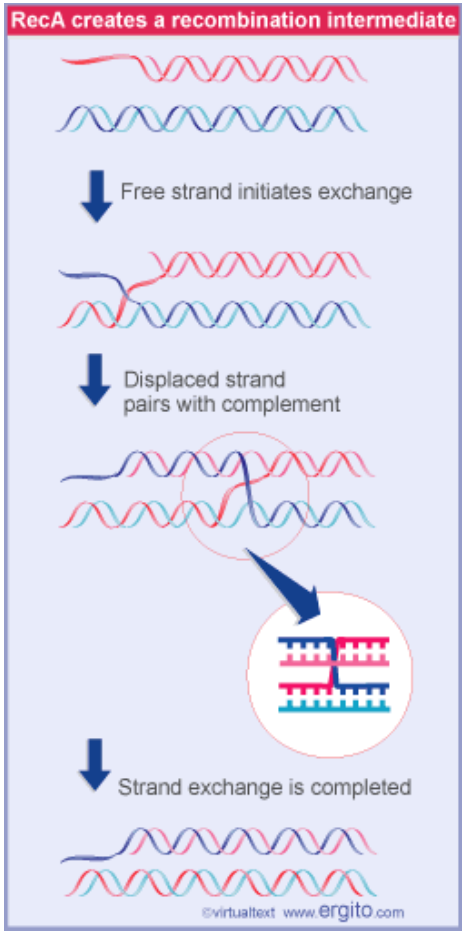
# RecA Creates Recombination Intermediates




RecA-mediated strand exchange between partially duplex and entirely duplex DNA generates a joint molecule with the same structure as a recombination intermediate.

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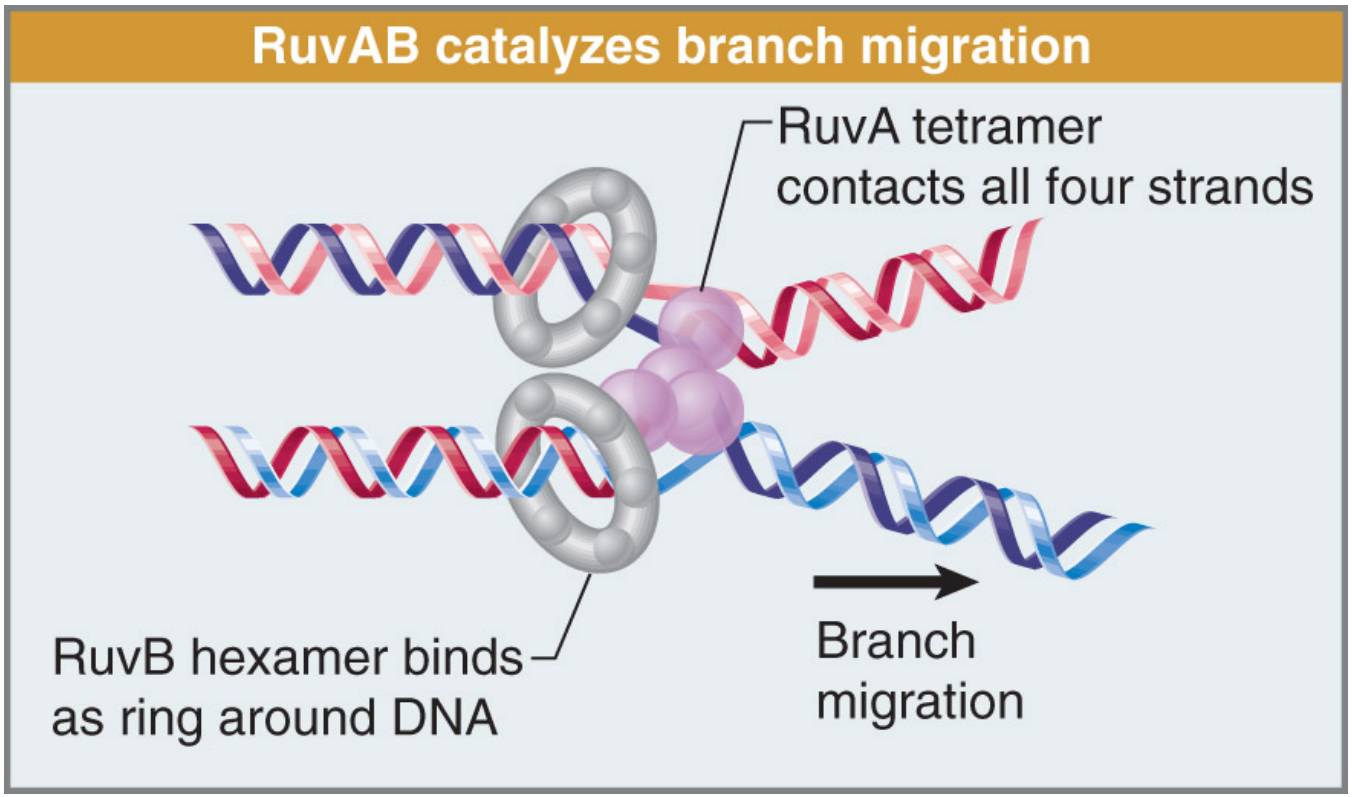
# The Ruv System Resolves Holliday Junctions

- The Ruv complex acts on recombinant junctions.

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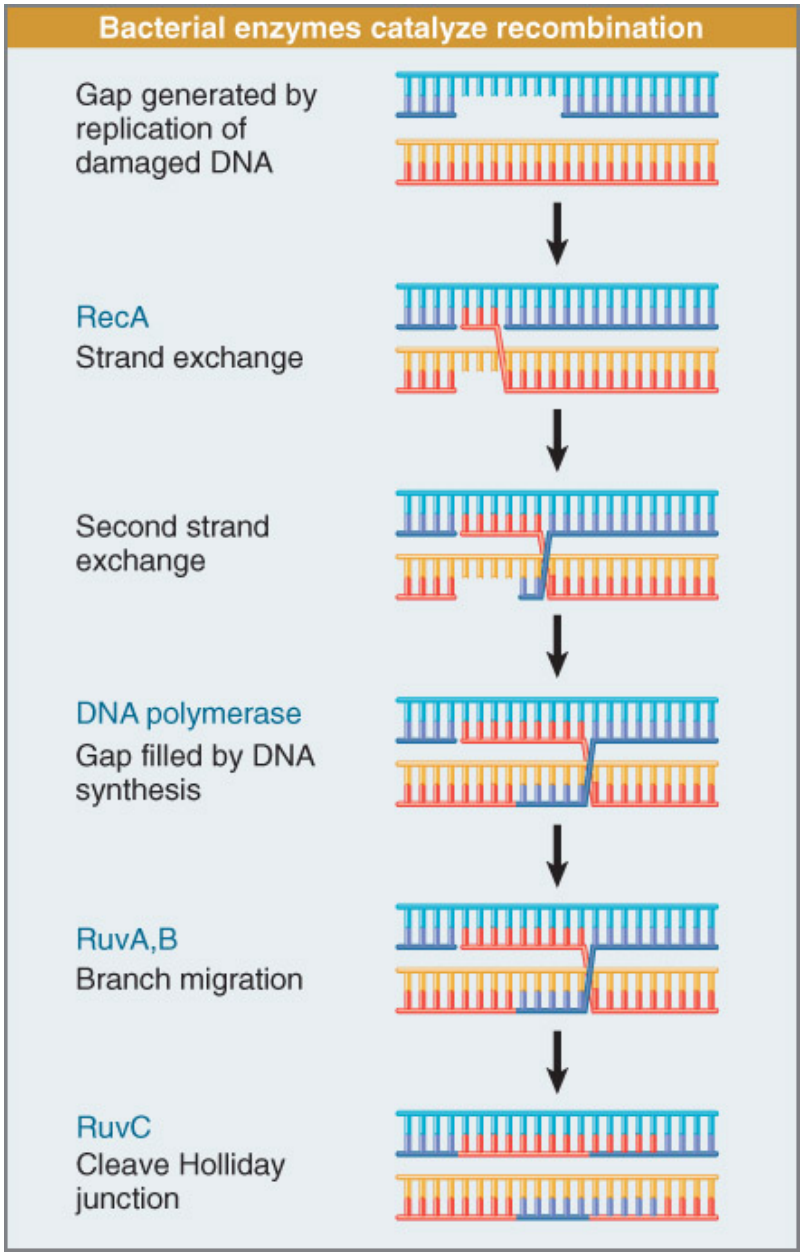
# Role of RuvABC in Recombination




- **RuvA** (27 kDa) recognizes the structure of the junction.
- **RuvB** (37 kDa) is a helicase that catalyzes branch migration.
- **RuvC** (19 kDa) cleaves junctions to generate recombination intermediates.

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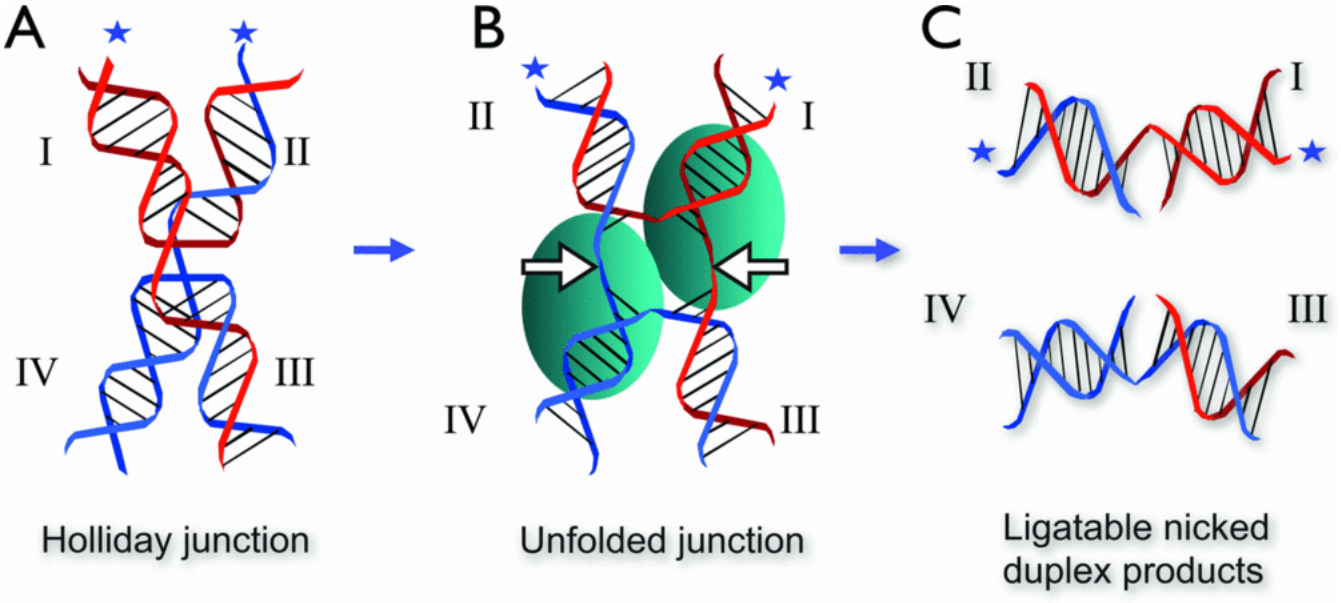
# Resolution of Holliday Junction




The continuous strands of the stacked-X structure (A) which form the wide angles in the unfolded RuvC-junction complex (B) are indicated by asterisks. The site of incision are indicated by arrows. The products of resolution (C) can be repaired by DNA ligase.

**Stephen C. West, Biochemical Society Transactions (2009) 37, 519-526**

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# Rad51 is a RecA Homolog in Eukaryotes

**Rad51**: catalyzes a synaptic reaction between a single strand DNA and a DNA double helix.

**Brca2**: interact with Rad51 and recruits Rad51 to ssDNA, facilitating strand exchange (Jensen et al., Nature doi:101038, 2010).

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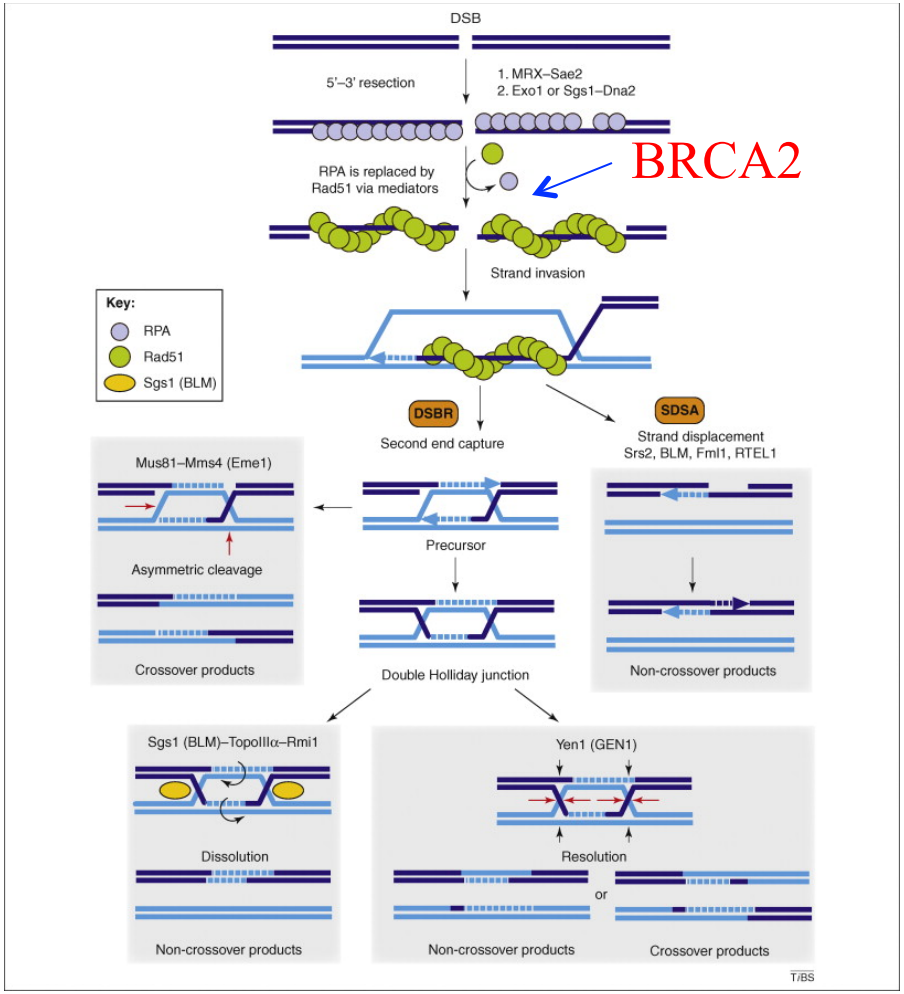
# Homologous Recombination Pathway




**Models and outcomes of the homologous recombination pathway.** 5′–3′ resection of the broken ends creates 3′ ssDNA tails that are rapidly coated by RPA (light blue). Rad51 (green) is recruited to the RPA-coated ssDNA by BRCA2, replacing RPA and forming the nucleoprotein filament, which can initiate pairing and strand invasion with the homologous duplex DNA. The 3′ end of the invading strand is extended by DNA synthesis using the donor duplex as a template. In the synthesis-dependent strand annealing (SDSA) model, the invading strand is displaced and pairs with the other 3′ single stranded tail, allowing DNA synthesis to complete repair. In the DSBR model, second end capture forms an early strand exchange intermediate. Processing of this precursor by Mus81–Mms4 (Eme1) generates crossover products, and ligation of this precursor creates a dHJ. Dissolution of the dHJ (via Sgs1 [BLM]–TopoIIIα-Rmi1) gives rise to non-crossover products, whereas resolution (via Yen1 [GEN1]) can lead to either crossover or non-crossover products.

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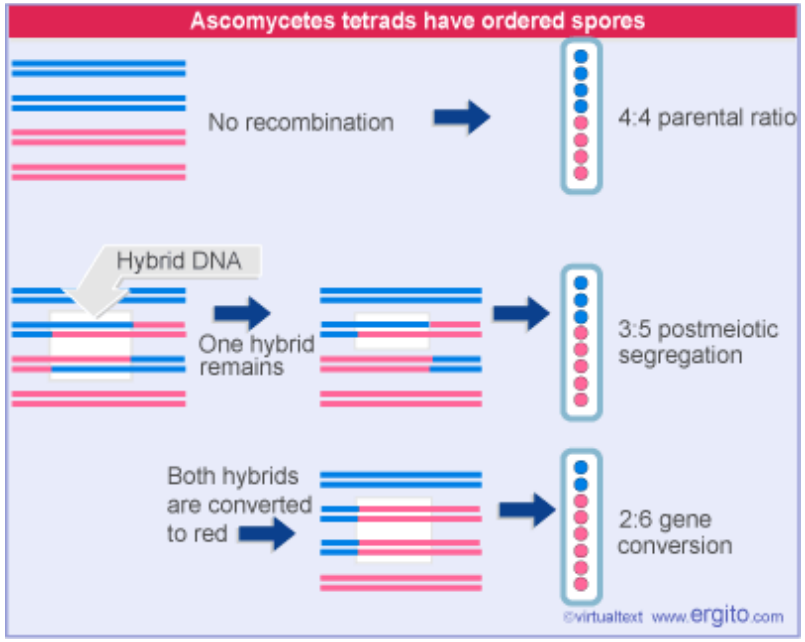
# Recombination Can Lead to Gene Conversion




**Key Terms**

•**Postmeiotic segregation** describes the segregation of two strands of a duplex DNA that bear different information (created by heteroduplex formation during meiosis) when a subsequent replication allows the strands to separate.

•**Gene conversion** is the alteration of one strand of a heteroduplex DNA to make it complementary with the other strand at any position(s) where there were mispaired bases.

**Key Concepts**

•Heteroduplex DNA that is created by recombination can have mismatched sequences where the recombining alleles are not identical.

•Repair systems may remove mismatches by changing one of the strands so its sequence is complementary to the other.

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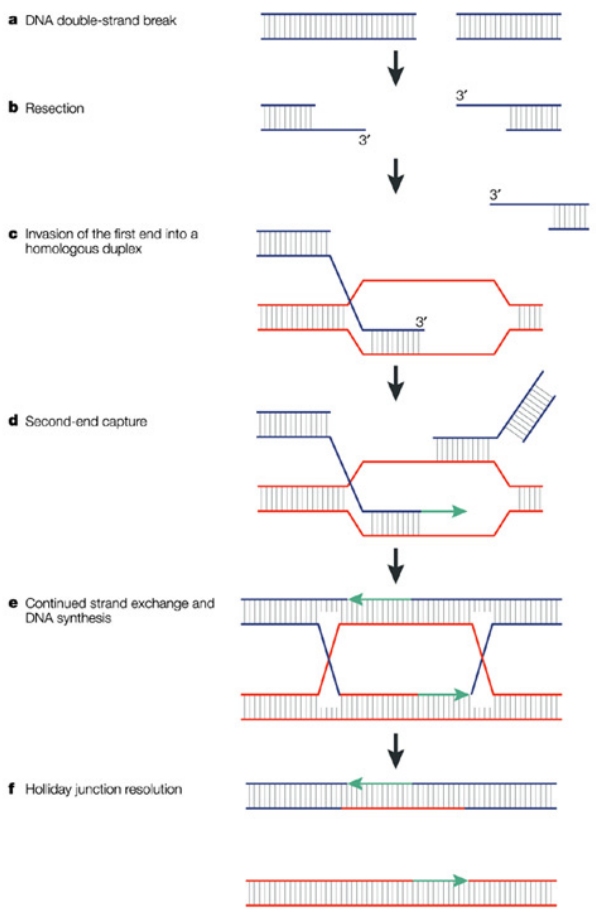
# Recombination Functions as a DNA Repair Mechanism

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DNA at the break site (**a**) is resected to expose single-stranded (ss)DNA (**b**). The resulting ssDNA becomes coated by the single-strand-binding protein, replication protein A (RPA), which in turn functions as a target for the binding of RAD52. As RAD51 interacts with RAD52, RAD51 can gain access to the ssDNA–RPA complex. (**c**) After assembly of an active nucleoprotein filament on the resected ssDNA tail of the first end, the complex pairs with homologous double-stranded (ds)DNA and strand exchange takes place. (**c**) In the next stage, RAD51 and/or RAD52 promote the capture of the second-end ssDNA tail. (**d** and **e**) The two invading ends function as primers for DNA resynthesis. (**f**) Finally, the DNA crossovers are resolved to allow the repaired duplexes to separate.

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# Summary

- Homologous recombination allows large section of the DNA to move from one chromosome to another; occurs only between homologous DNA molecules.

- Homologous recombination begins with a double-strand break that is created by Spo11 and processed by RecBCD to expose a single-stranded DNA end;

- Synapsis is catalyzed by RecA protein, and leads to the formation of a Holliday junction;

- Resolution of the Holliday junction is conducted by RuvABC.

- Homologous recombination can lead to gene conversion and repair double strand DNA breaks.

# DNA Recombination

**DNA recombination**:

- General recombination (homologous recombination)
- Site-specific recombination

# Site-Specific Recombination

• Site-specific recombination, also called specialized recombination, can alter gene order and add new information to the genome.

• Site-specific recombination moves specialized nucleotide sequences, called mobile genetic elements, between nonhomologous sites within a genome.

• Site-specific recombination occurs by two different mechanisms:

1. Transpositional site-specific recombination
2. Conservative site-specific recombination

# Transposition

**Key Terms**

**Transposons** (also called transposable elements):
Discrete sequences in genome that are mobile.

**Transposase**:
An enzyme encoded by the transposon and disconnecting the transposon from one piece of DNA and inserting it into a new target DNA site.

# Barbara McClintock & Transposon

Barbara McClintock (June 16, 1902 – September 2, 1992), the 1983 **Nobel Laureate** in Physiology or Medicine.

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