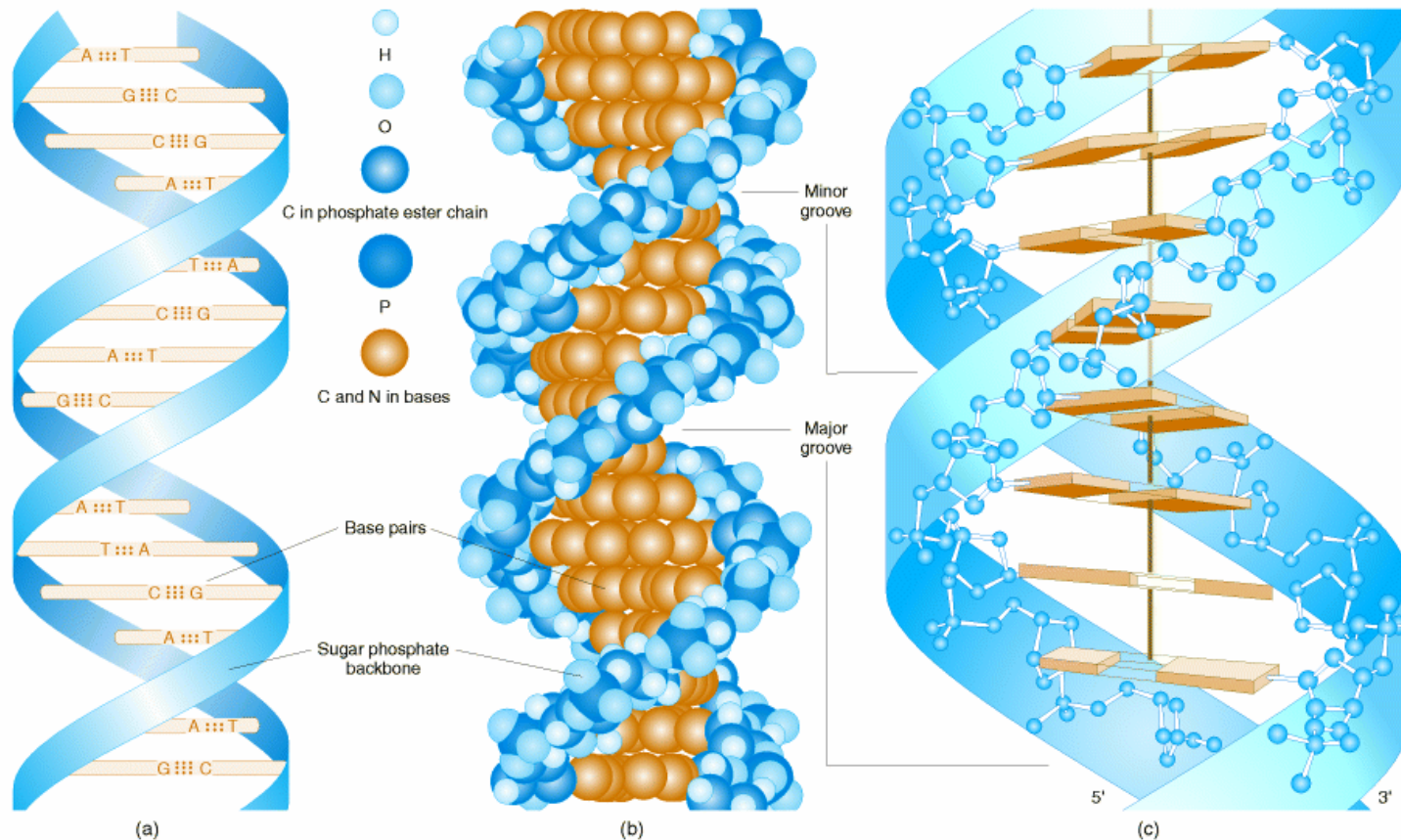
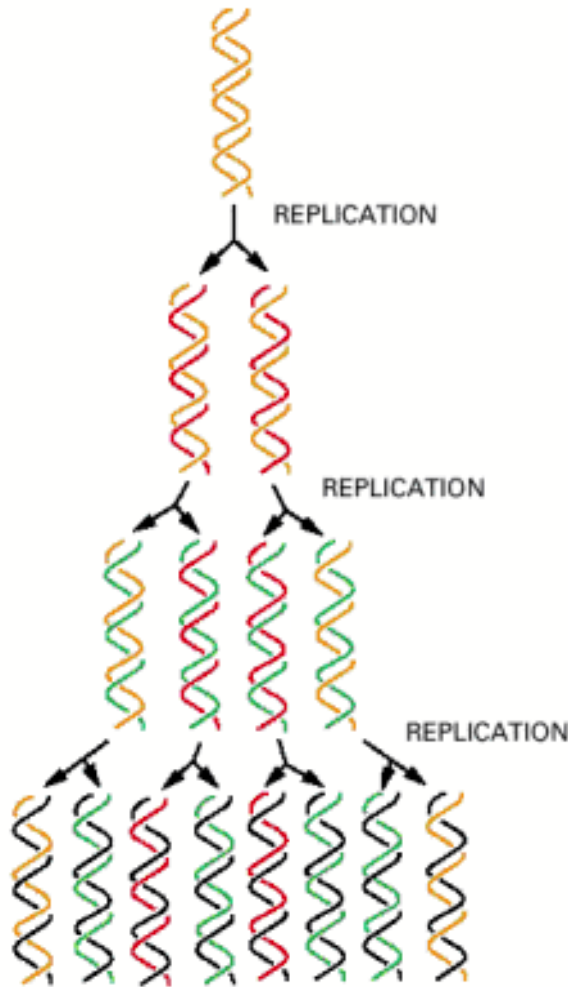


# Three representations of the DNA double helix

- **Double helix:** 2 associated polynucleotide strands wind together to form a helix
- **Anti-parallel:** The orientation of the 2 strands are anti-parallel; their 5'→3' directions are opposite
- **Complementary:** 2 polynucleotide strands maintain Watson-Crick base pairs (A-T, C-G) which results in two strands that are complementary to each other



# DNA Replication is Semi-conservative



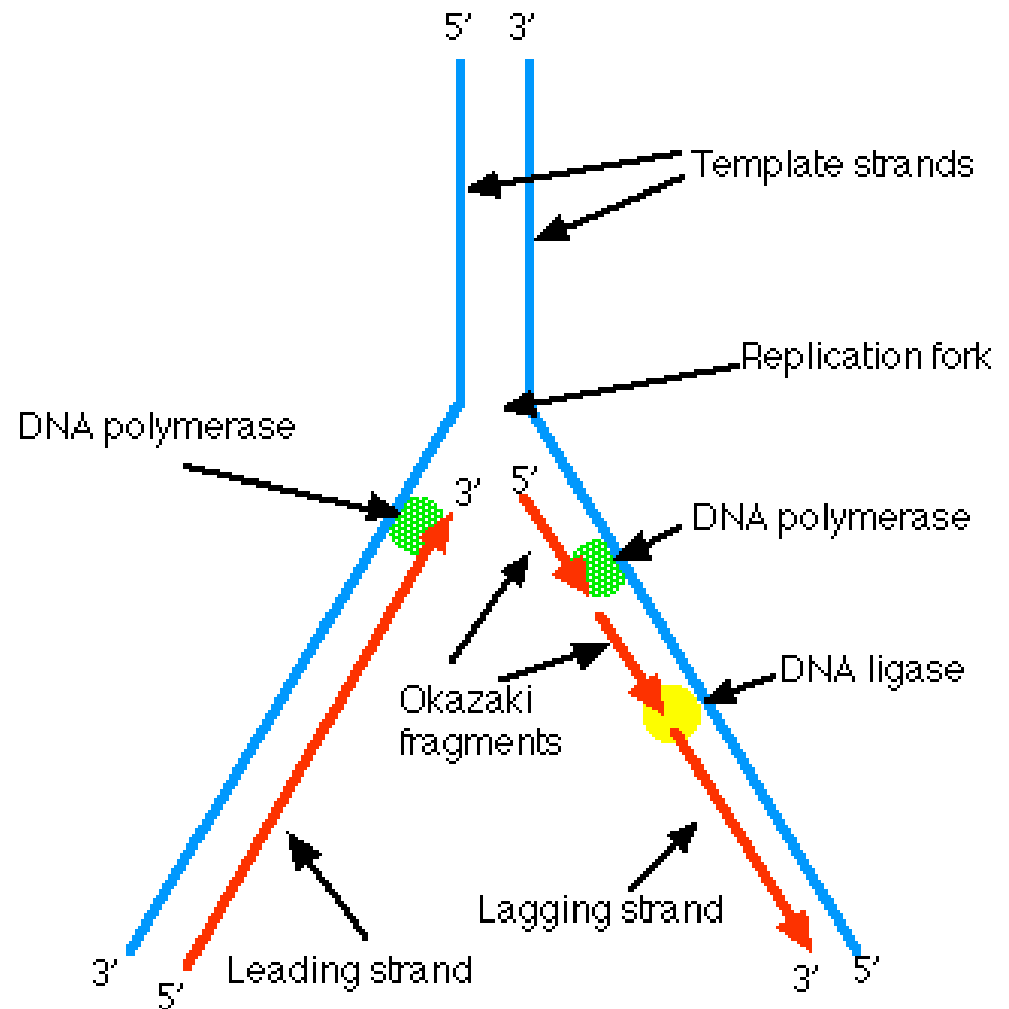
One half of each new DNA molecule is old, the other half new.

This model was first proposed by Watson and Crick, but wasn't proven until experiments done by Meselson and Stahl.

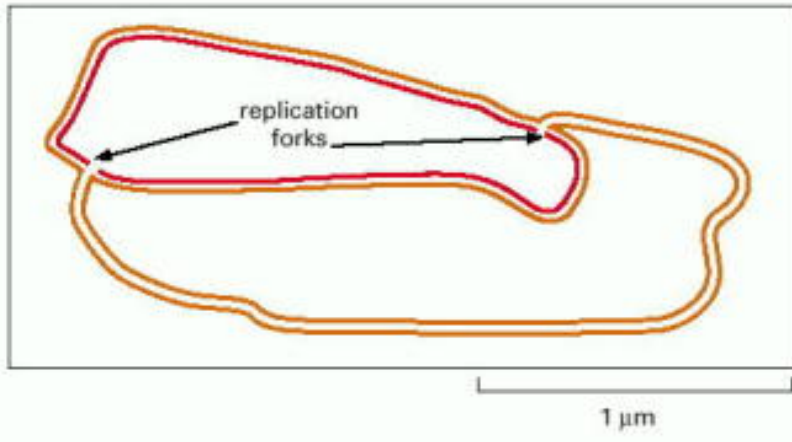
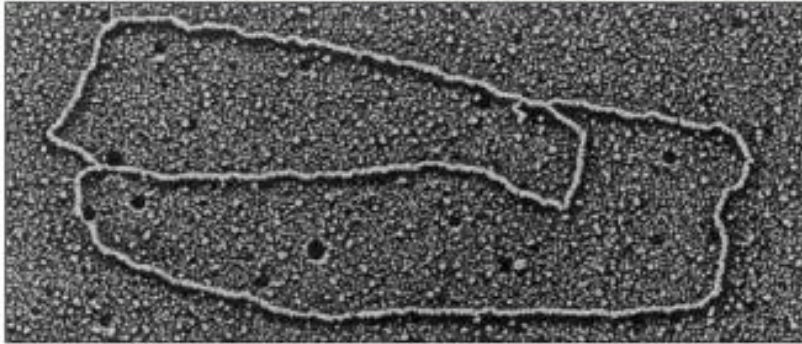


# Model of DNA Replication

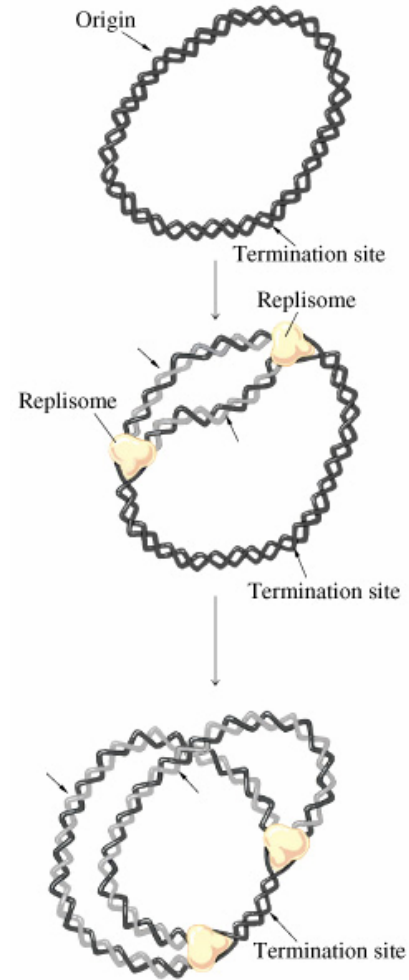
[Animation](#)



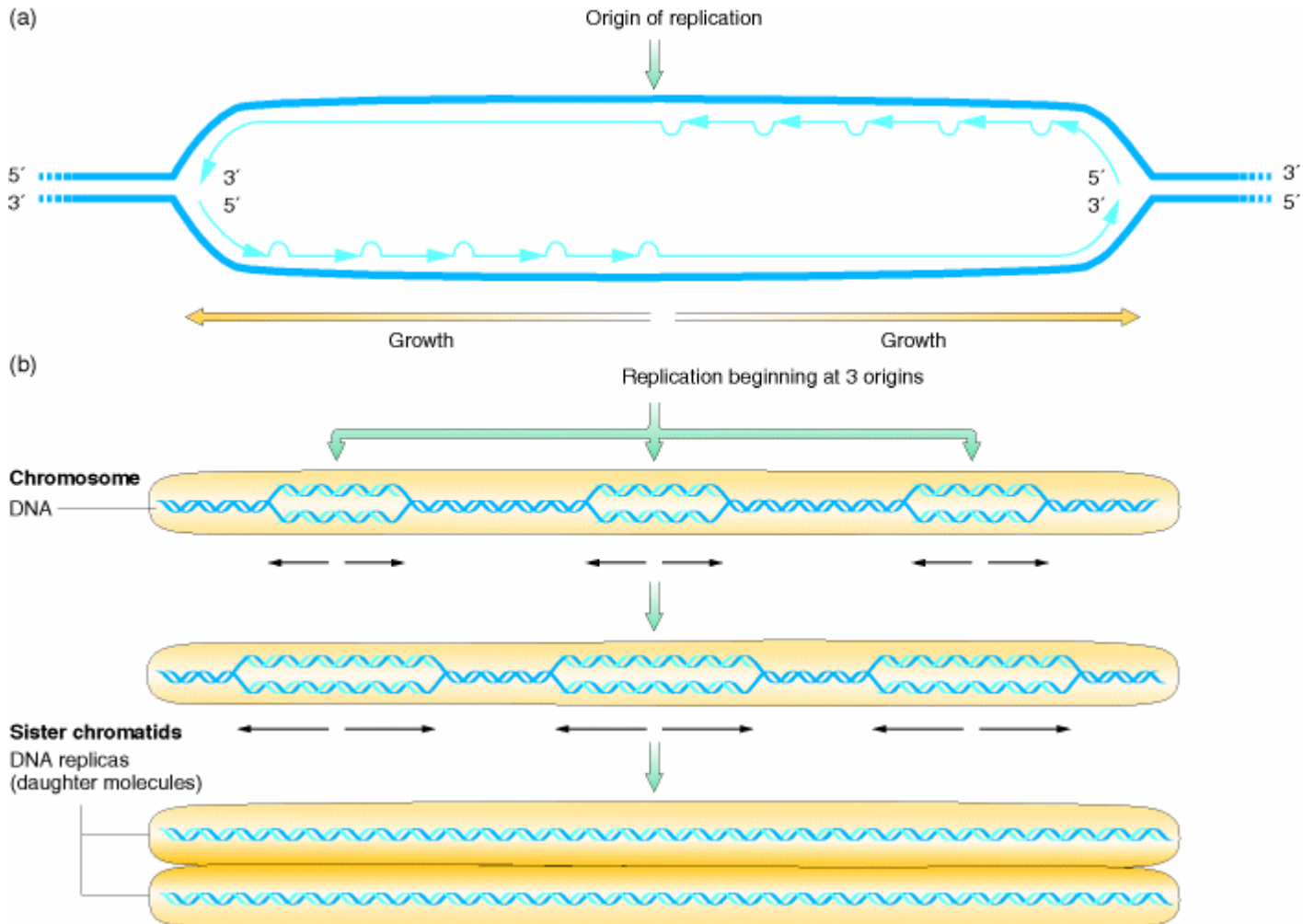
# Prokaryotic Replication



The 2 replication forks move in opposite directions on a circular chromosome.



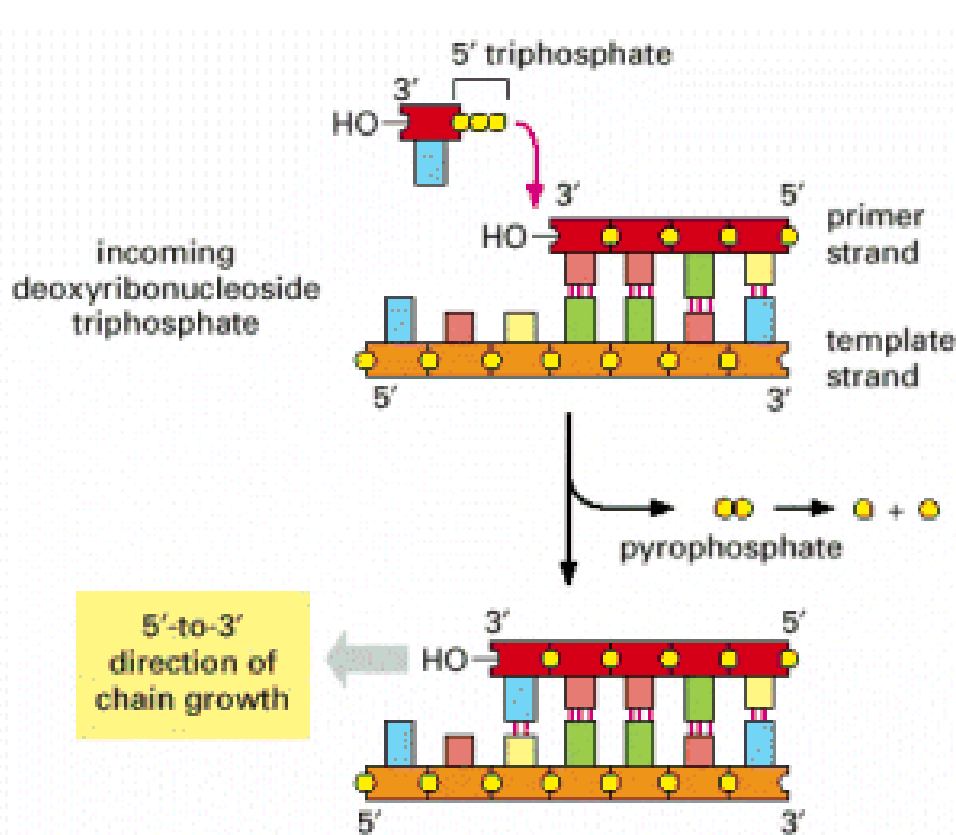
# Eukaryotic Replication: Formation of Bubbles



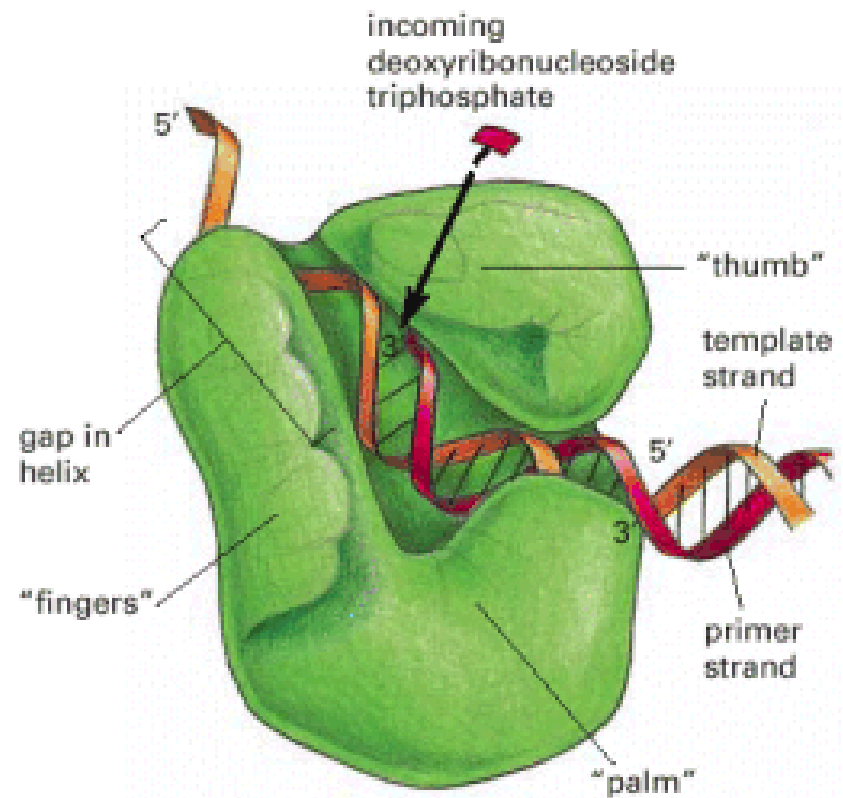
[Animation](#)

Bi-directional growth of the replication fork causes the formation of bubbles in the DNA. Near the end of replication, the bubbles fuse, resolving the 2 molecules of DNA.

# DNA Synthesis is Carried Out by DNA Polymerase

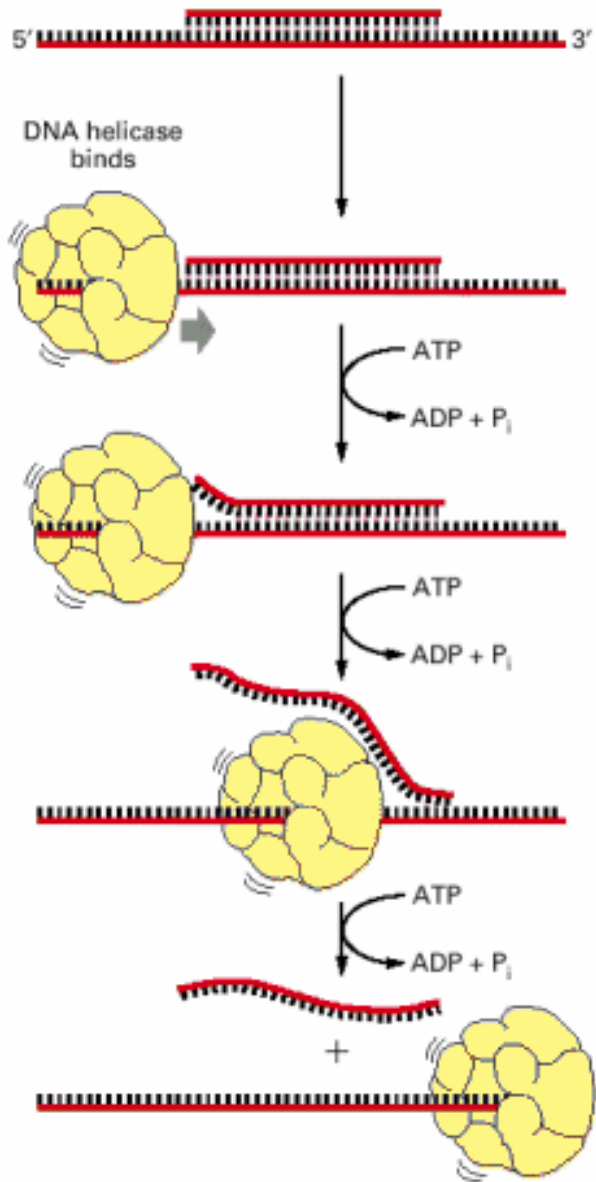


(A)



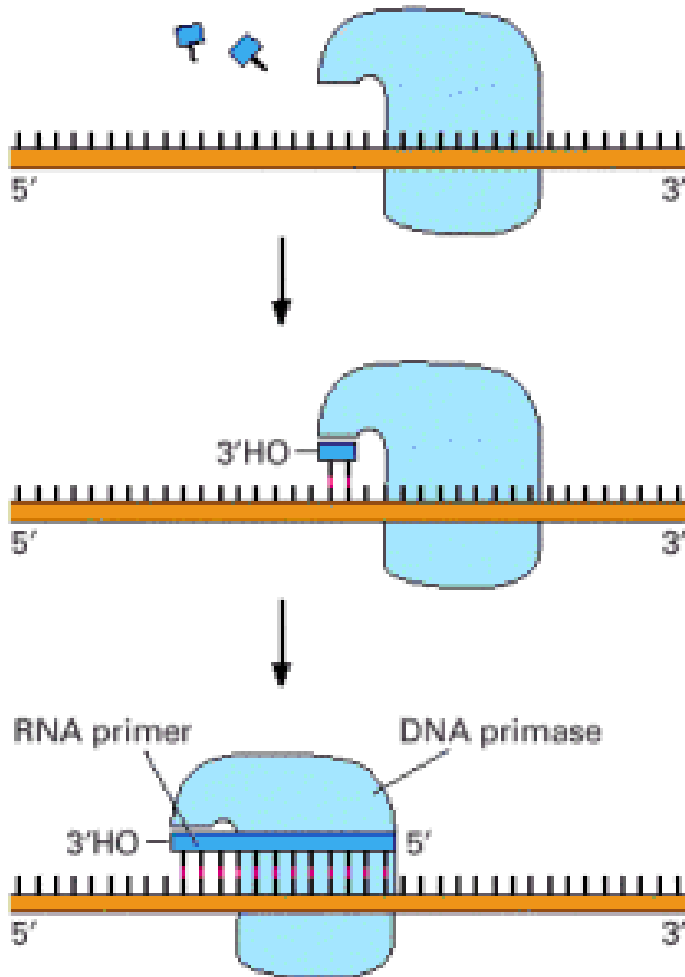
(B)

# Helicase: Unwinding the Duplex



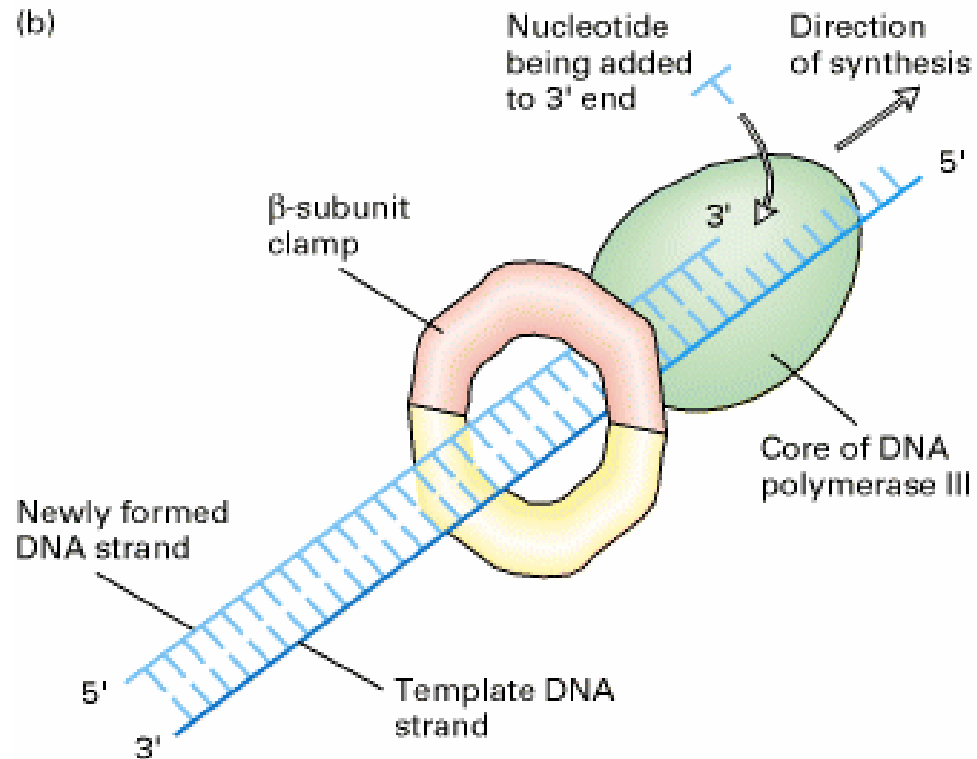
Helicase breaks hydrogen bonds in DNA and unwinds it during movement of the replication fork.

# Primase: Getting Replication Started



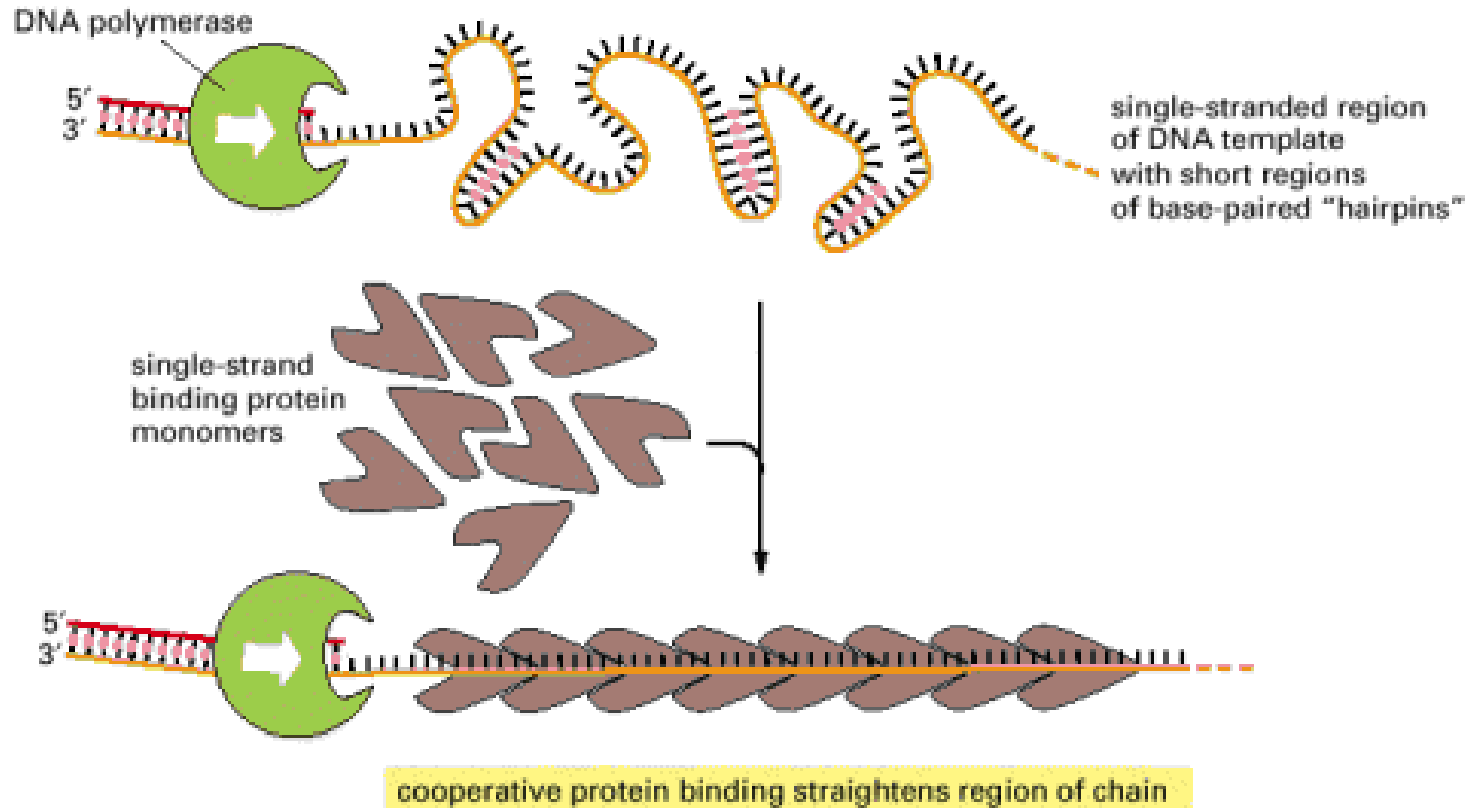
DNA Polymerase can't start replicating on it's own. It needs DNA Primase to start by synthesizing a short **RNA Primer** in the 5' → 3' direction, making the 3' end available for DNA Polymerase.

# $\beta$ -clamp: Holding things together



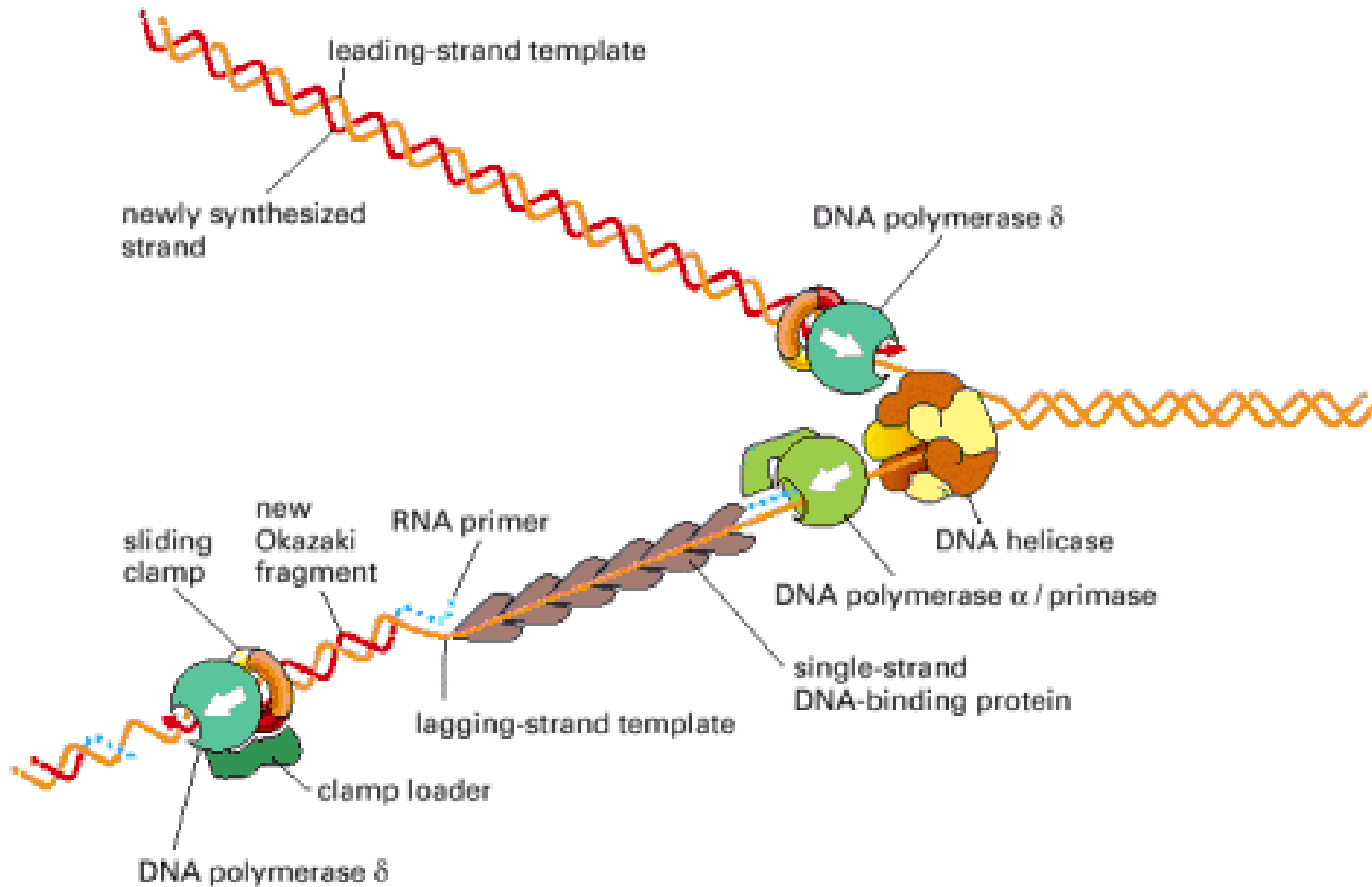
The clamps hooks up with the DNA Polymerase, an interaction which keeps the core from falling off the template.

# SSBP: Getting the wrinkles out



SSB proteins help to straighten out the DNA in front of the DNA Polymerase, helping to pave the way for the replication machinery. They also keep the unpaired DNA strands from reannealing during the replication process.

# The Replication Fork



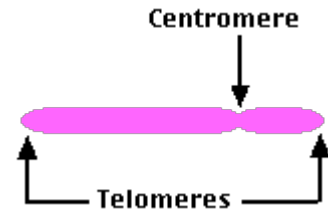
# Telomeres

Telomeres: specialized ends of eukaryotic (linear) chromosomes

The DNA molecule of a typical chromosome contains a linear array of genes (encoding proteins and RNAs) interspersed with much noncoding DNA.

Included in the noncoding DNA are:

- long stretches that make up the **centromere** and
- long stretches at the ends of the chromosome, the **telomeres**.

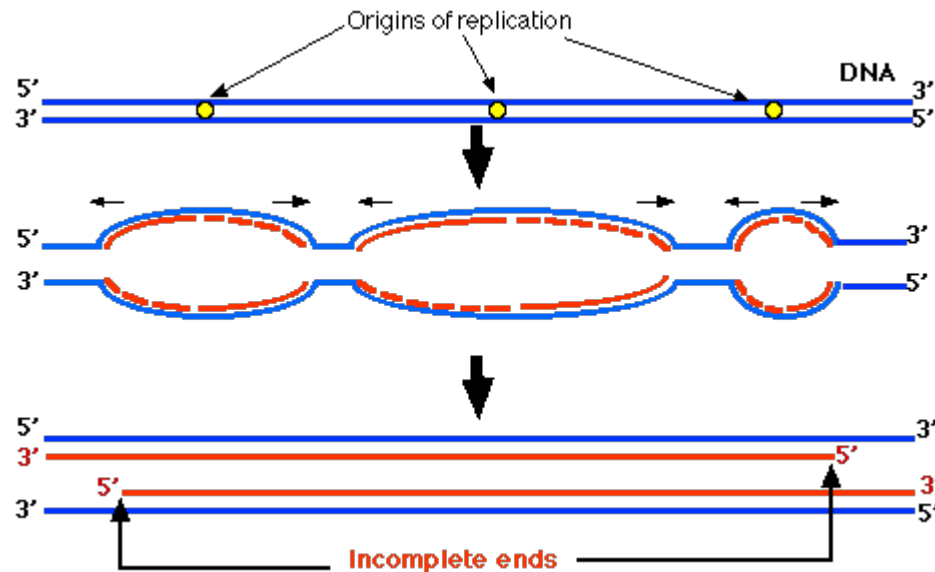


Telomeres are crucial to the life of the cell. They keep the ends of the various chromosomes in the cell from accidentally becoming attached to each other.

The telomeres of humans consist of as many as 2000 repeats of the sequence:  
5' **TTAGGG** 3'.

5'...TTAGGG TTAGGG TTAGGG TTAGGG TTAGGG TTAGGG..3'  
3'...AATCCC AATCCC AATCCC AATCCC AATCCC AATCCC..5'

# Telomeres: A Replication Challenge



As the replication fork nears the end of the DNA, there is no longer enough template to continue forming Okazaki fragments. So the 5' end of each newly-synthesized strand cannot be completed.

Thus each of the daughter chromosomes will have a shortened telomere.

It is estimated that human telomeres lose about 100 base pairs from their telomeric DNA at each mitosis.

This represents about 16 TTAGGG repeats. At this rate, after 125 mitotic divisions, the telomeres would be completely gone.

# Telomerase Allows Replication of Telomeres

Telomerase is an enzyme that adds telomere repeat sequences to the 3' end of DNA strands. By lengthening this strand DNA polymerase is able to complete the synthesis of the "incomplete ends" of the opposite strand. [Animation](#)

Telomerase:

- is a **ribonucleoprotein**;
- Its single RNA molecule provides an AAUCCC (in mammals) template to guide the insertion of TTAGGG.
- Its protein component — called hTERT in humans ("human TElomere Reverse Transcriptase") — provides the catalytic action.
- Thus telomerase is a **reverse transcriptase**; synthesizing DNA from an RNA template.

Telomerase is generally found only in

- the cells of the germline, including embryonic stem (ES) cells;
- unicellular eukaryotes like *Tetrahymena thermophila*;
- cancer cells.

However, some human cells have been shown to briefly express telomerase during S phase of their cell cycle (a time when new chromosomes are being synthesized). Perhaps all our cells do.

# Accuracy of Replication Very Important

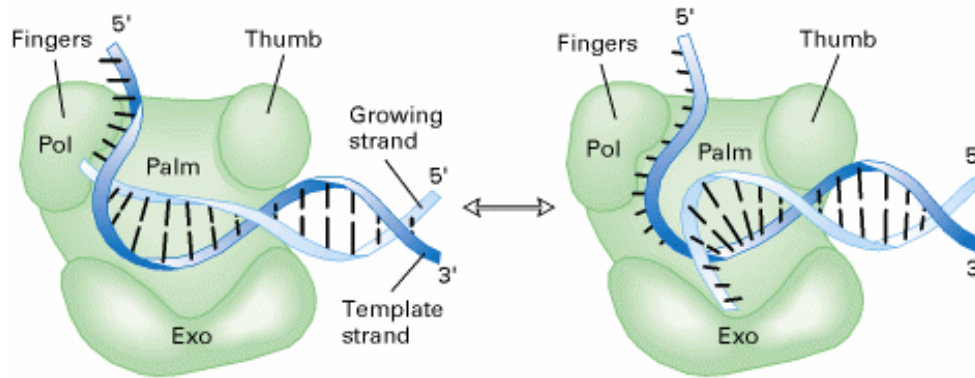
A number of human diseases are due to mistakes that can be traced to errors in DNA replication. A few examples include:

- Fragile X syndrome
- X-linked spinal and bulbar muscular atrophy (known as *Kennedy disease*)
- Myotonic dystrophy (main cause of adult muscular dystrophy)

Most of these diseases are caused due to “slippage” in DNA replication of highly repetitive sequences.

# Proofreading by DNA Polymerase

DNA Polymerases do make mistakes during replication, but thankfully they have a proofreading function which is able to correct most of these mistakes.



When an incorrect base is incorporated during DNA synthesis,

1. The DNA polymerase pauses,
2. Then transfers the 3' end to the exonuclease site where the mismatched base is removed.
3. Then the 3' end is transferred back to the polymerase site,
4. Where this region is copied correctly.

# Other types of DNA Damage

DNA in the living cell is subject to many chemical alterations. If the genetic information encoded in the DNA is to remain uncorrupted, any chemical changes must be corrected.

**A failure to repair DNA produces a mutation.**

Examples of agents that cause DNA damage:

- Radiation: such as UV rays, X-rays and Gamma rays
- Chemicals in the environment: such as hydrocarbons found in cigarette smoke
- Oxidative radicals: highly reactive, produced by normal cell function

Examples of types of DNA Damage:

- Direct modification of bases
- Mismatches
- Strand Breaks
- Crosslinks

# DNA Repair Mechanisms

Damaged or inappropriate bases can be repaired by several mechanisms:

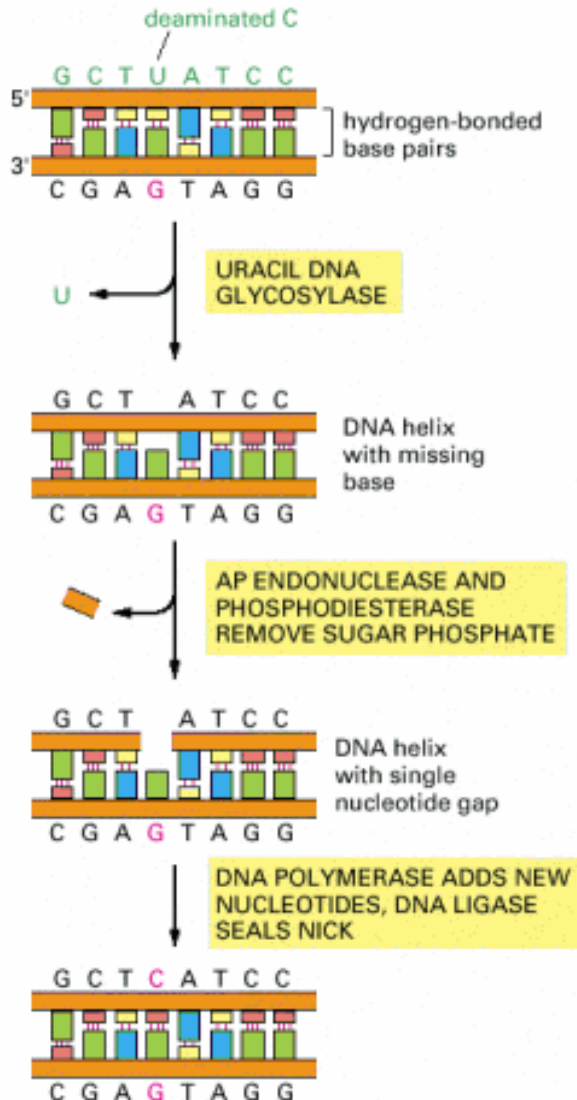
- **Direct chemical reversal** of the damage
- **Excision Repair**, in which the damaged base or bases are removed and then replaced with the correct ones in a localized burst of DNA synthesis.

Three modes of excision repair, each of which employs specialized sets of enzymes.

- **Base Excision Repair**
- **Nucleotide Excision Repair**
- **Mismatch Repair**

# Base Excision Repair

## (A) BASE EXCISION REPAIR

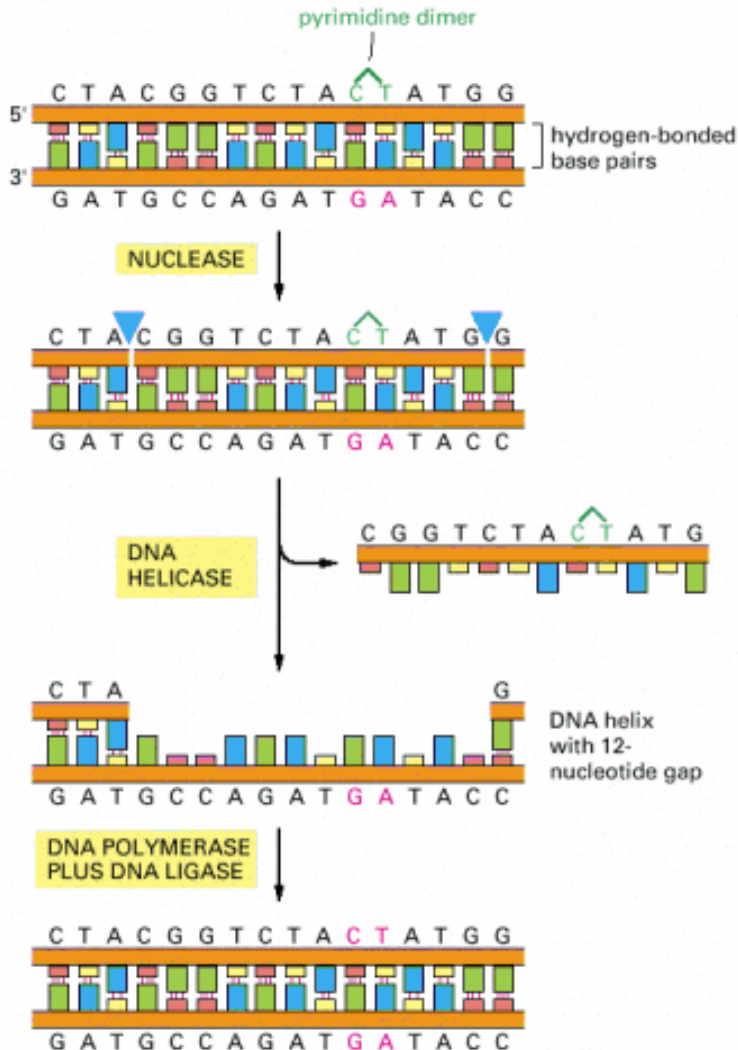


The steps and some key players:

- Removal of the damaged base by a DNA glycosylase.
- Removal of its deoxyribose phosphate in the backbone, producing a gap.
- Replacement with the correct nucleotide.
- Ligation of the break in the strand.

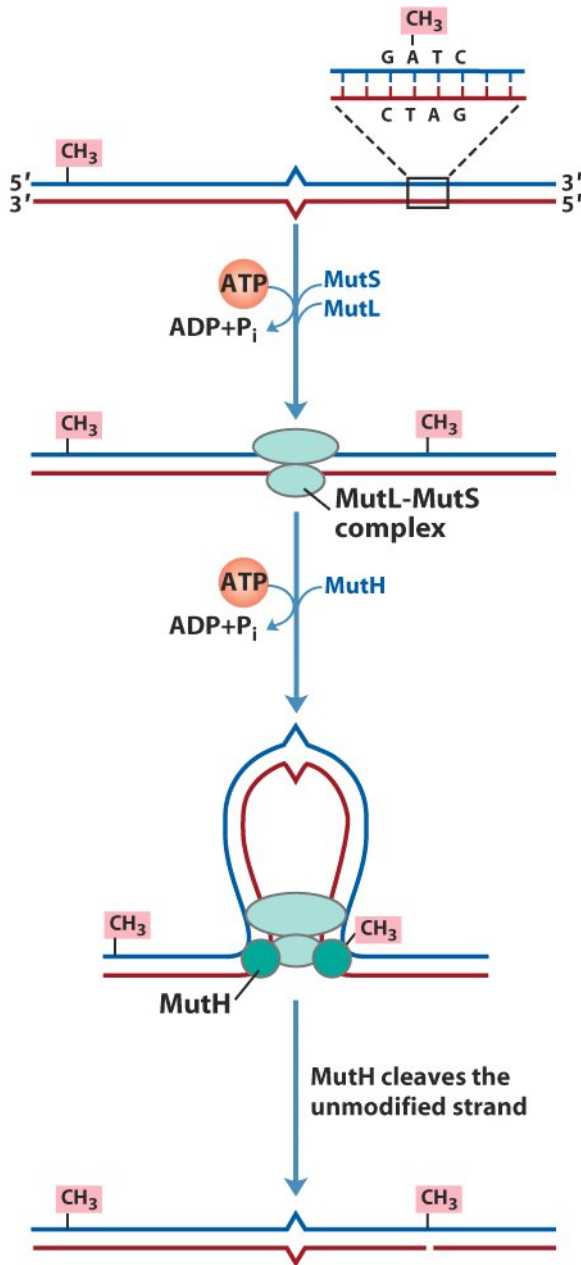
# Nucleotide Excision Repair

## (B) NUCLEOTIDE EXCISION REPAIR



The steps and some key players:

- The damage is recognized by one or more protein factors that assemble at the location.
- The DNA is unwound producing a "bubble".
- Cuts are made on both the 3' side and the 5' side of the damaged area so the tract containing the damage can be removed.
- A fresh burst of DNA synthesis — using the intact (opposite) strand as a template — fills in the correct nucleotides.
- A DNA ligase covalent binds the fresh piece into the backbone.



# Mismatch Repair

Mismatch Repair deals with correcting mismatches of normal bases.

Steps in MMR:

- **Recognition of a mismatch**
- **Identification of newly synthesized strand**
- **Removal of mismatch**
- **Gap repair by DNA Pol**

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