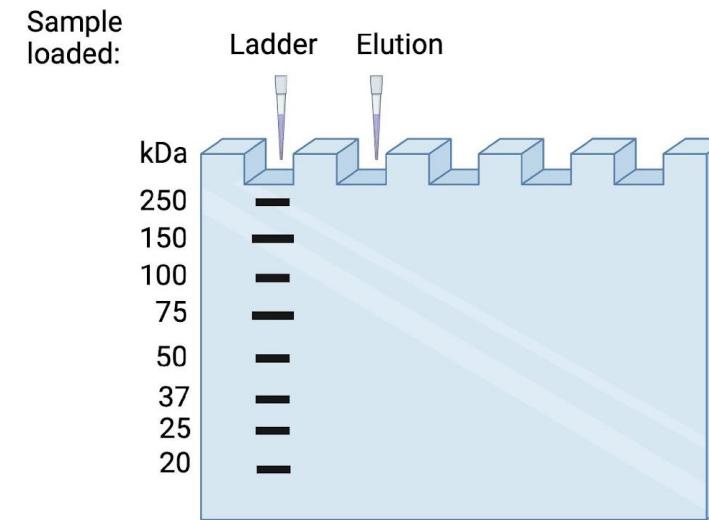
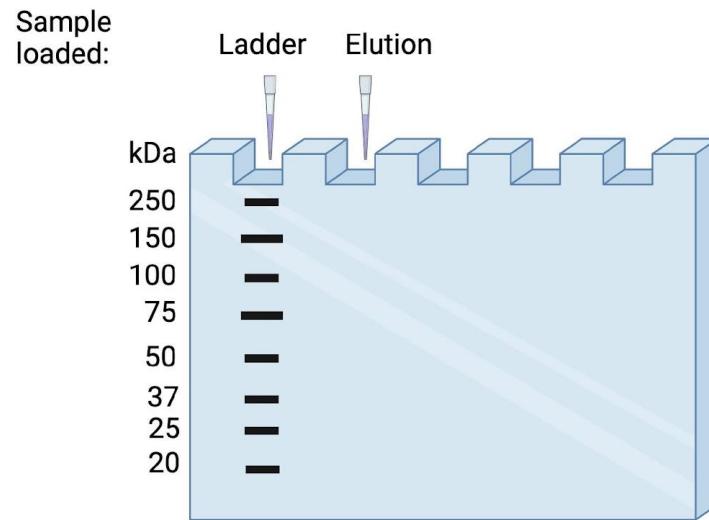


Review: Analyzing protein structure

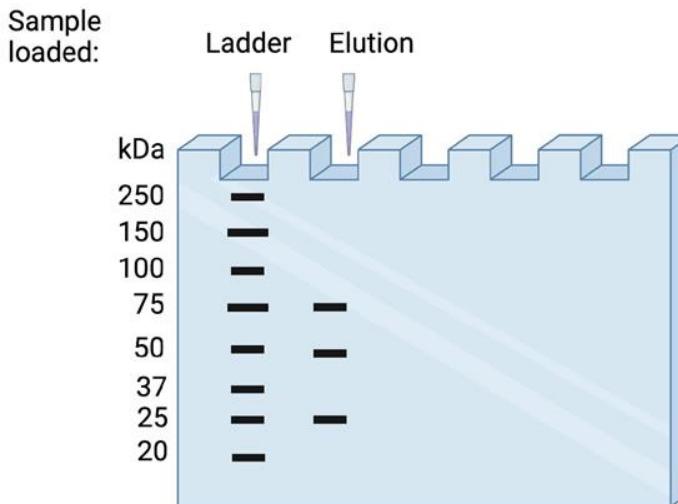
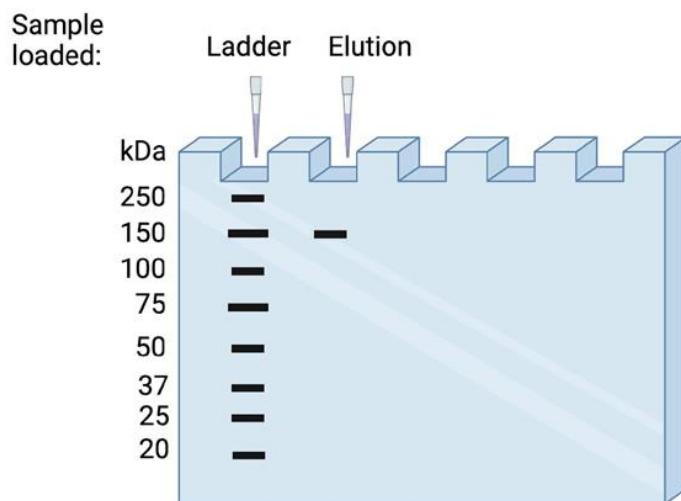
- Protein Z has 3 subunits, each linked by disulfide bonds. They are sized as 25 kDa, 50 kDa, and 75 kDa.
- On the left gel, draw what result you would expect to see if you add SDS but do not add a reducing agent such as DTT or β -mercaptoethanol, which break disulfide bonds.
- On the right gel, draw what result you would expect to see if you add both SDS and DTT.



- Protein Z is a highly positively charged protein, in your first attempt running the gel you forgot to add SDS and obtained a weird result showing your protein as close to 250 kDa, why might this be?

Review: Analyzing protein structure

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- Protein Z is a highly positively charged protein, in your first attempt running the gel you forgot to add SDS and obtained a weird result showing your protein as close to 250 kDa, why might this be?
 - Gel electrophoresis relies on charged electric current to move proteins towards a positively charged anode, and like charges repel. Without SDS, which coats proteins in a large negative charge, protein Z will not process towards the anode at the speed anticipated from its size and will move more slowly, or even towards the negatively charged cathode.



Chapter 5&6: Chromosomes, DNA Replication & Repair

Dr. Matthew Ellis

Learning Objectives for Chapter 5 & 6:

Ch 5:

- Identify experiments that led to the discovery of DNA as the hereditary molecule
- Understand the structure of DNA in terms of the double helix shape
- Identify how eukaryotic DNA packs into chromosomal structures and describe how chromosome structure regulates gene expression

Ch 6:

- Understand the steps of DNA replication in eukaryotic cells and describe the multiple roles of DNA polymerase and other proteins at the replication fork
- Identify causes of DNA damage in cells and describe how DNA repair mechanisms can fix damaged DNA

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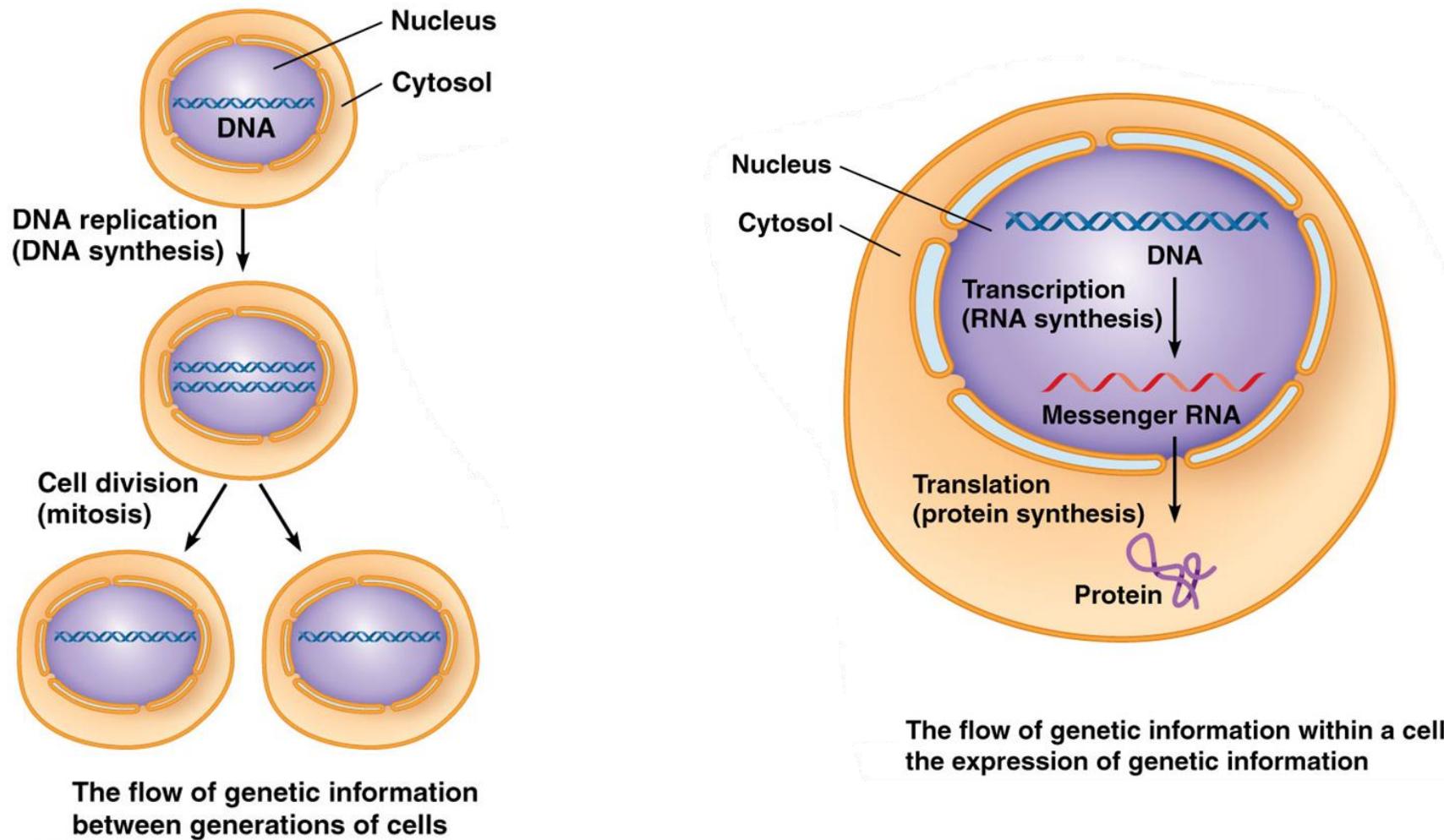
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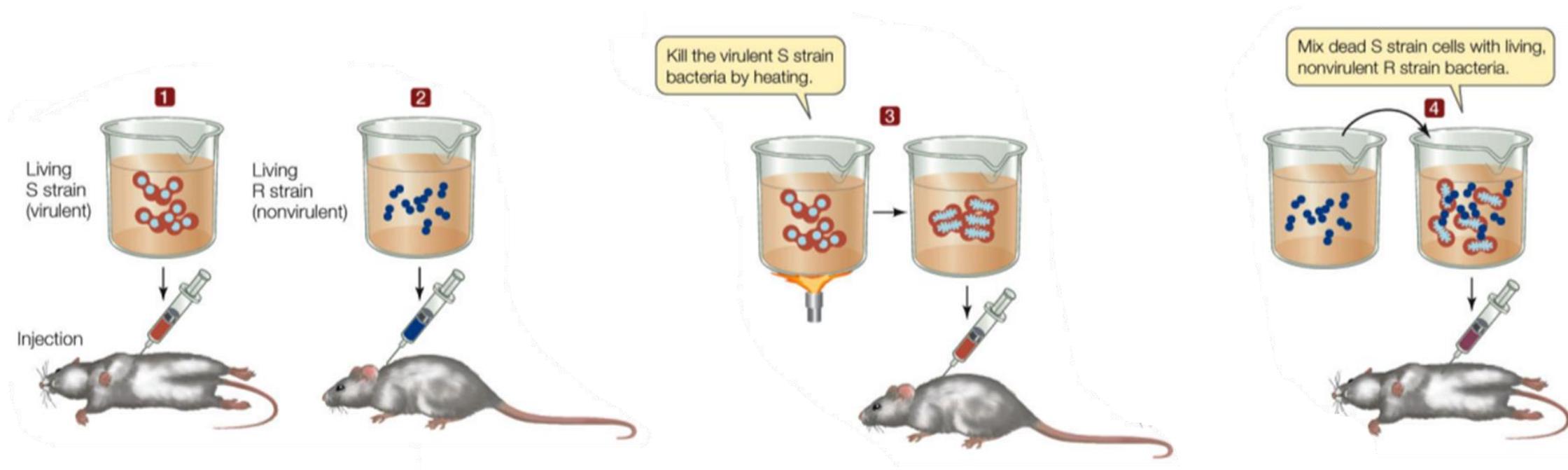
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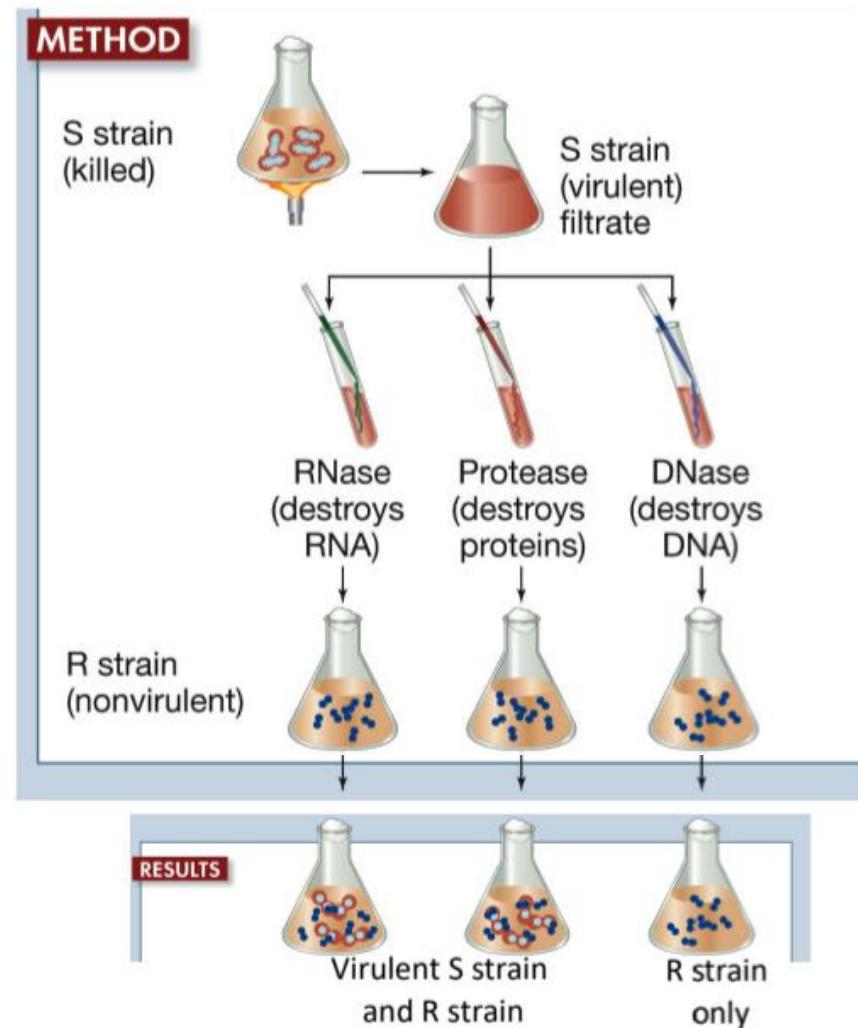
DNA exists in our cell nuclei and serves as the master code for gene expression



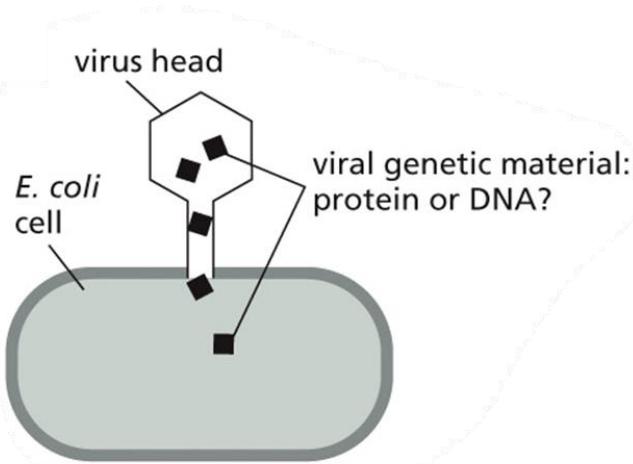
Griffith (1928) demonstrated that a substance prepared from dead cells can add a heritable trait to living cells



Avery, MacLeod, McCarty (1944) shows it was DNA not RNA or protein that carries heritable traits



Hershey & Chase (1952) further confirmed that DNA is injected by viruses into cells as the heritable unit of life



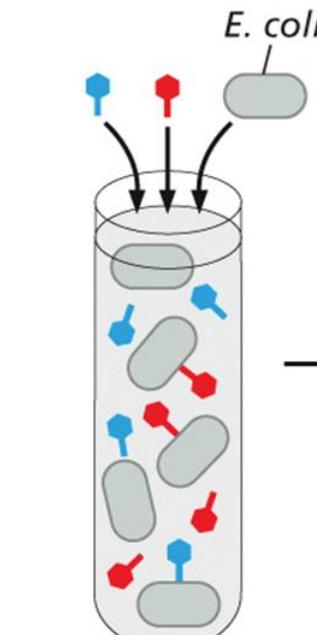
Phosphorus
found only in
DNA not protein

DNA labeled
with ^{32}P

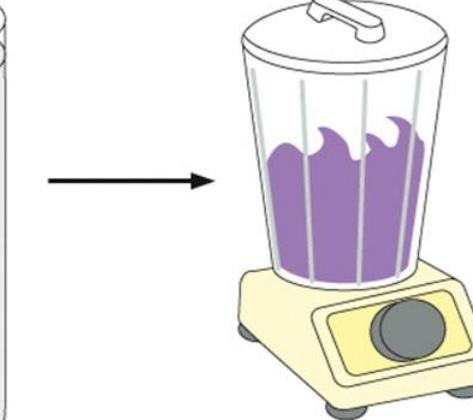


protein labeled
with ^{35}S

Sulfur found only
in protein not DNA



viruses allowed to
infect *E. coli*



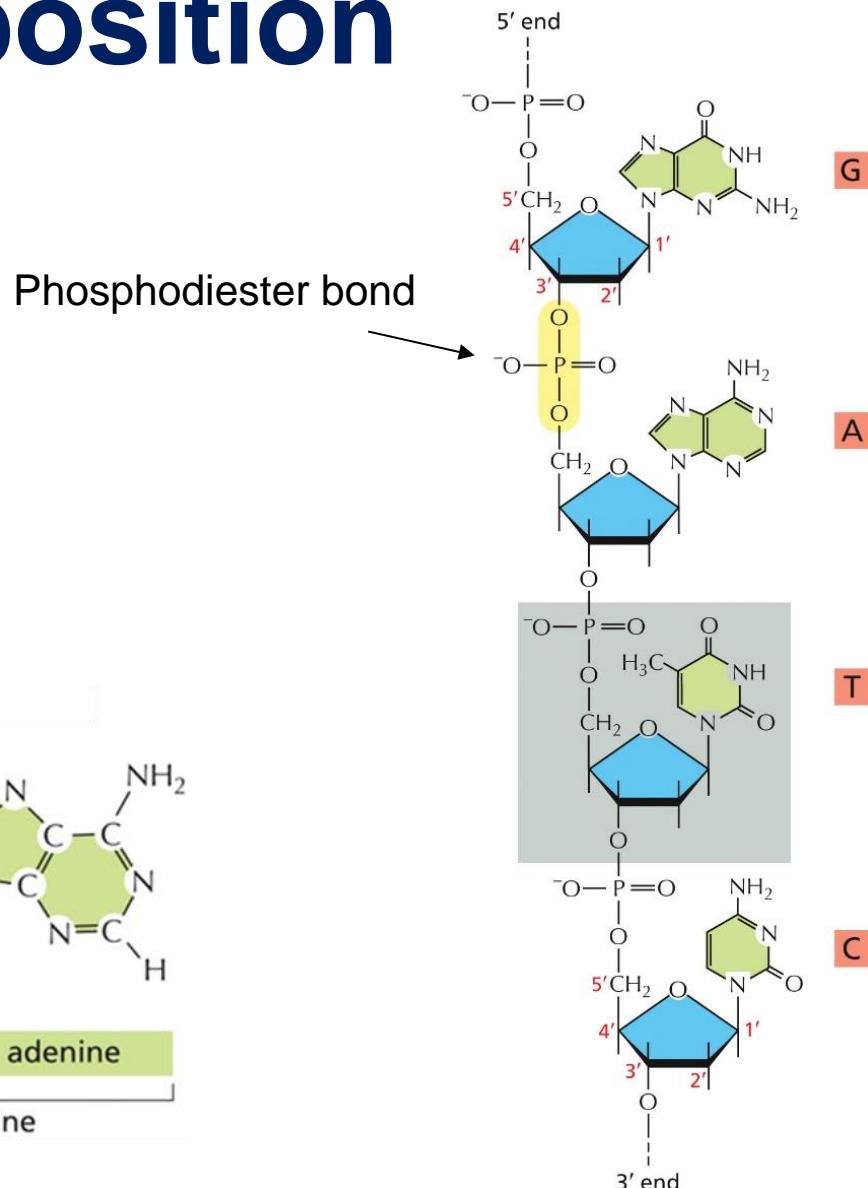
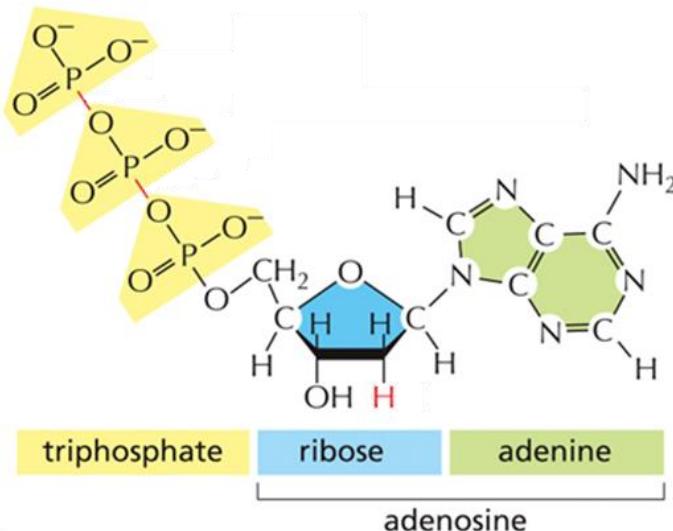
viral heads
sheared off
the bacteria



infected bacteria
contain ^{32}P but
not ^{35}S

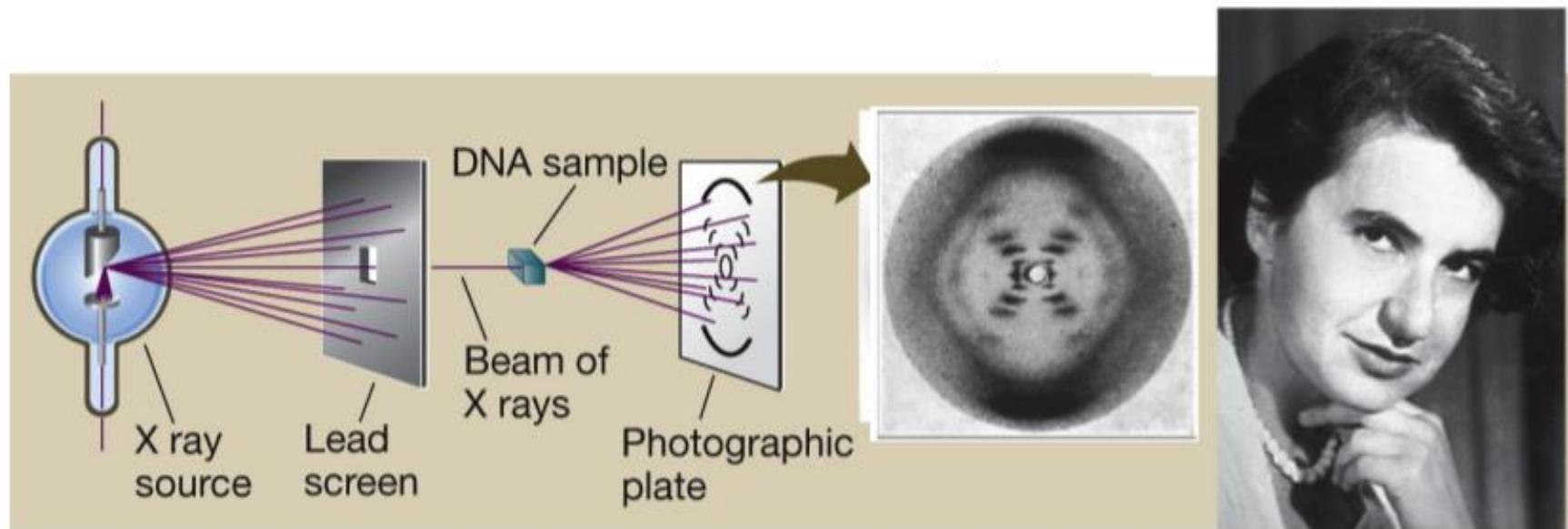
Recall: Nucleic acid composition

- DNA is built from **nucleotides**
 - Consist of nitrogen containing ring compound (nitrogenous base) linked to a five-carbon sugar (deoxyribose) with one or more phosphate groups attached to it
 - Each nucleotide is named after the base it contains (A,T,C,G)

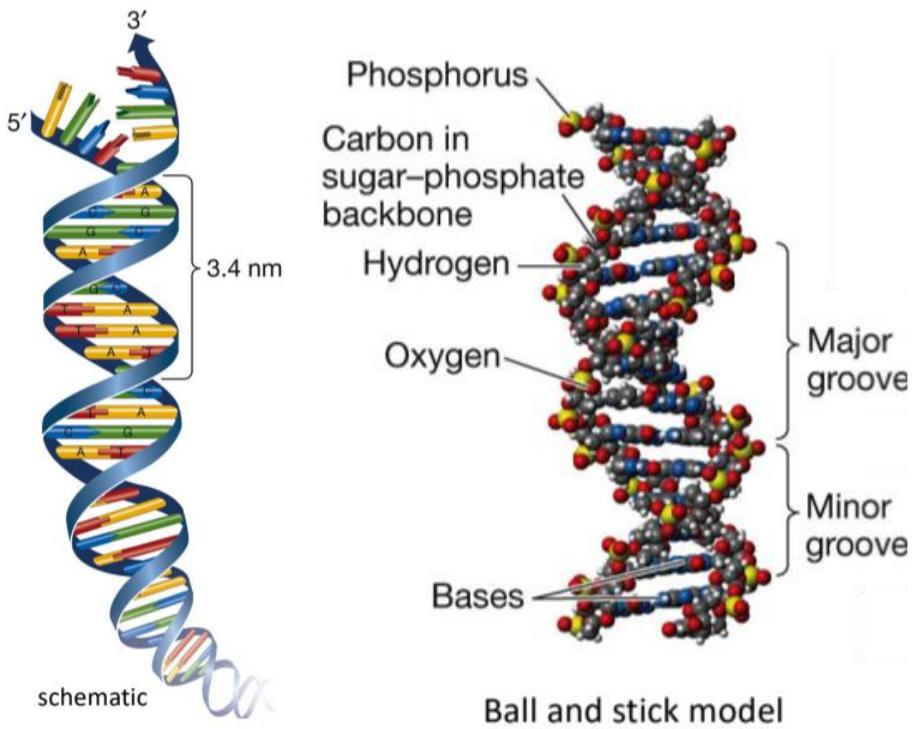


DNA chemical composition was known,
but not its shape and structure

Rosalind Franklin takes x-ray diffraction image hinting at structure of DNA fiber



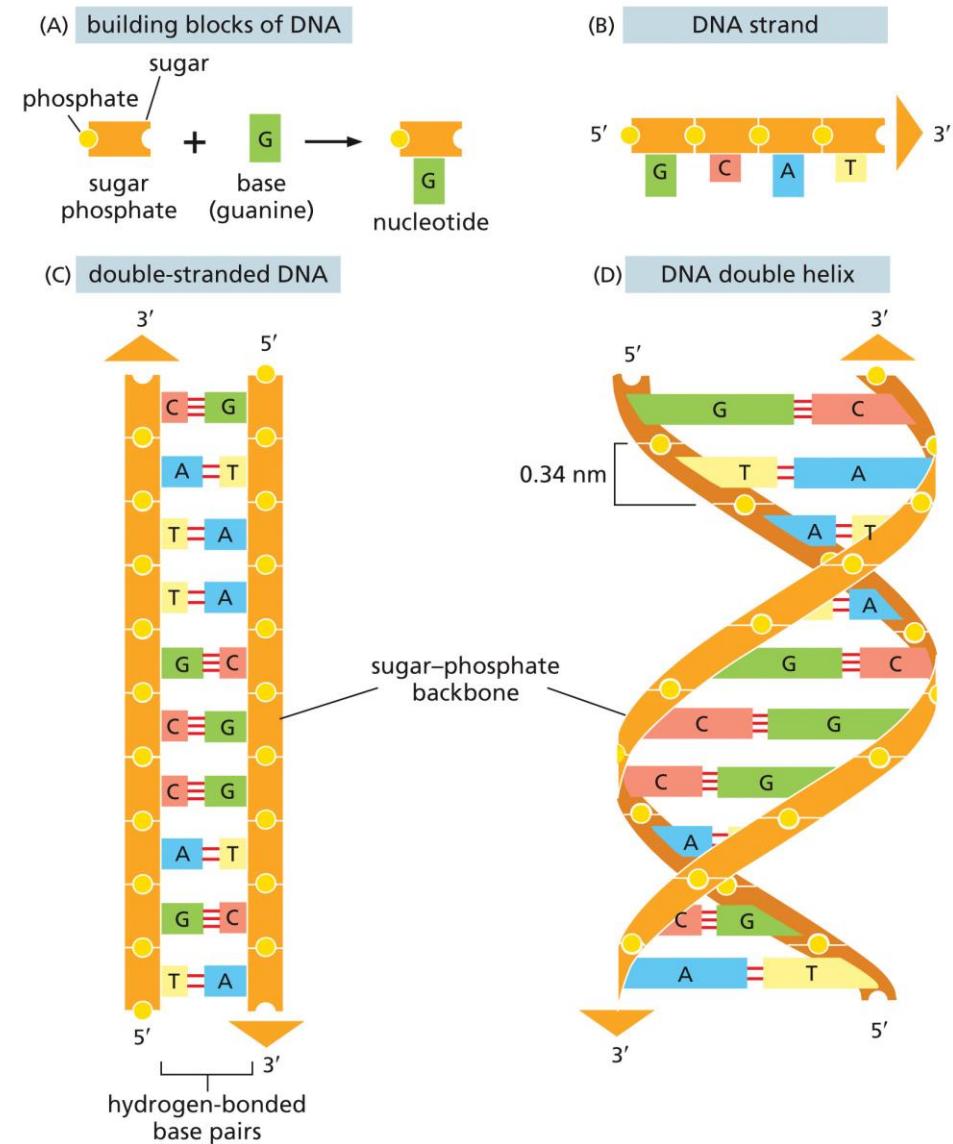
Watson & Crick construct the *double helix* model of DNA (1953)



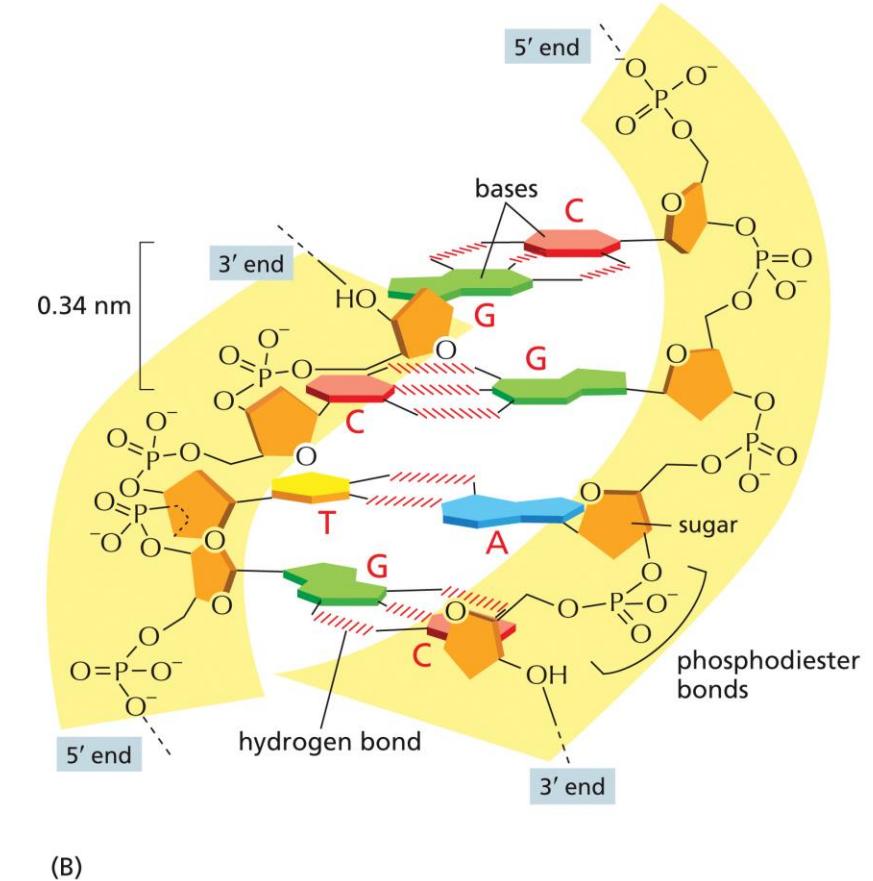
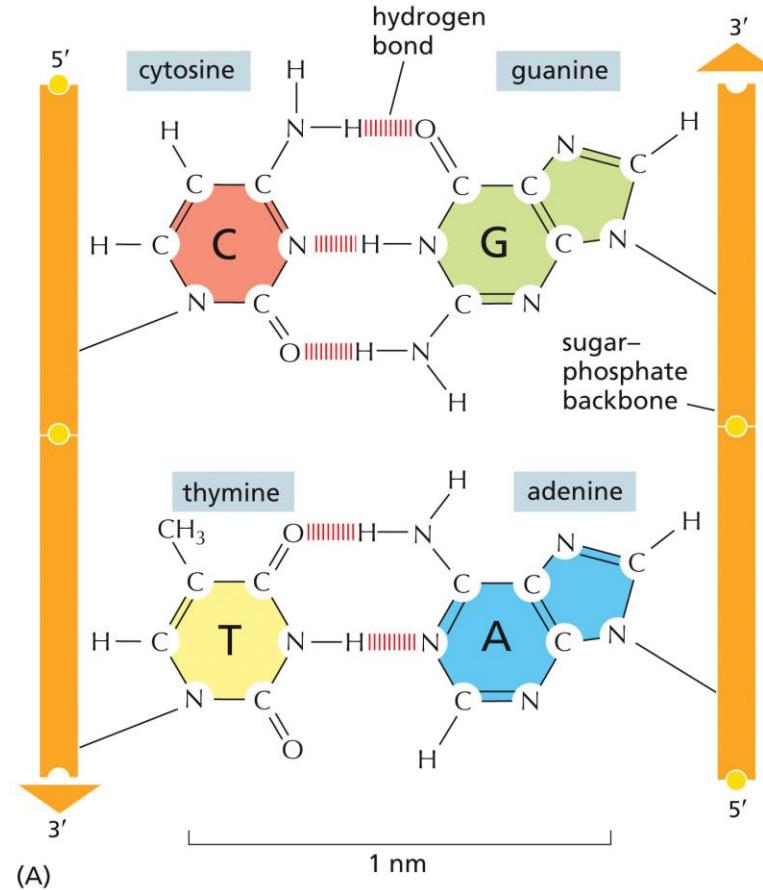
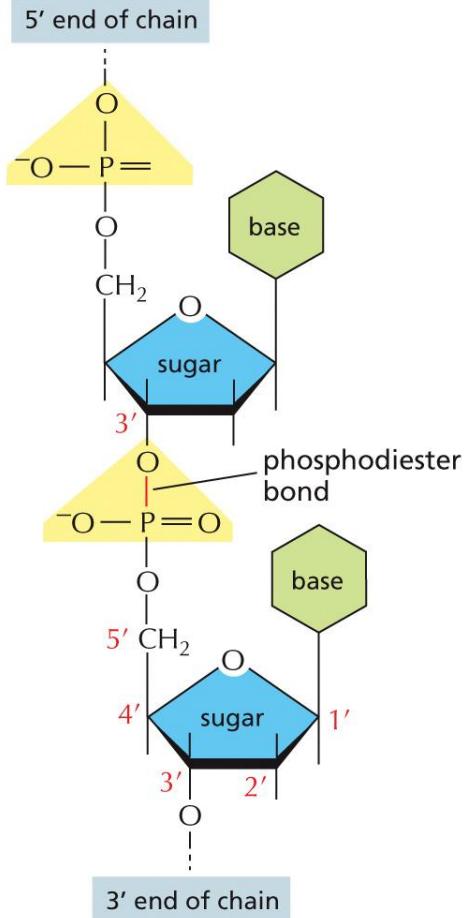
- DNA strands are *antiparallel* meaning they display opposite polarity
- Hydrophobic bases stacked in center, away from aqueous environment
- Negatively charged phosphates on outside to interact beneficially with water

DNA double helix model

- Twisting is energetically favorable
- Two polynucleotide chains consisting of purines (A, G) and pyrimidines (C, T) held together by hydrogen bonds for complementary base pairing
 - A=T
 - G=C
- Phosphodiester bonds form from 5' → 3'



Complementary base pairing and stacking



Squarecap #1-2

Learning Objectives for Chapter 5 & 6:

Ch 5:

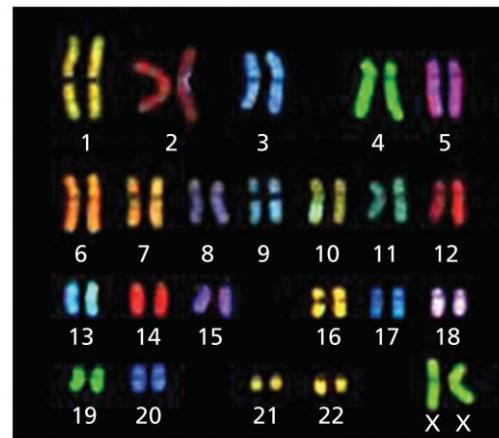
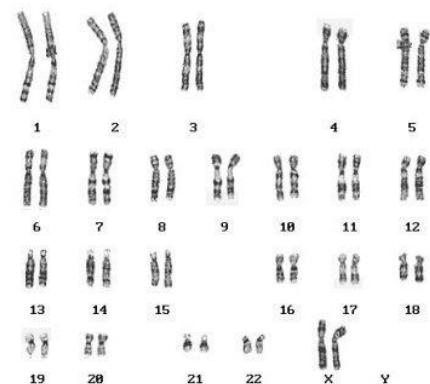
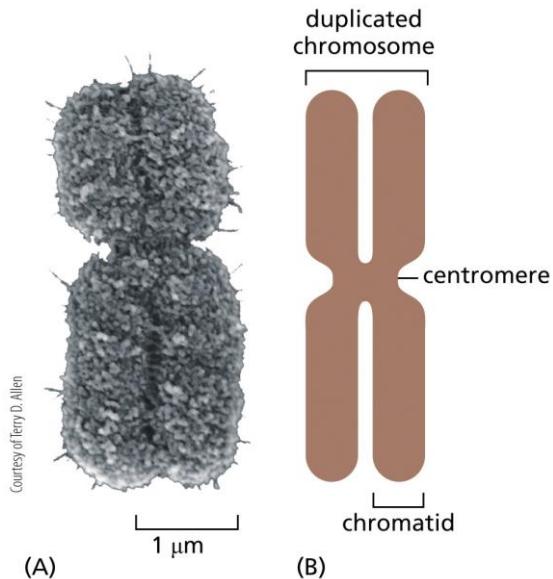
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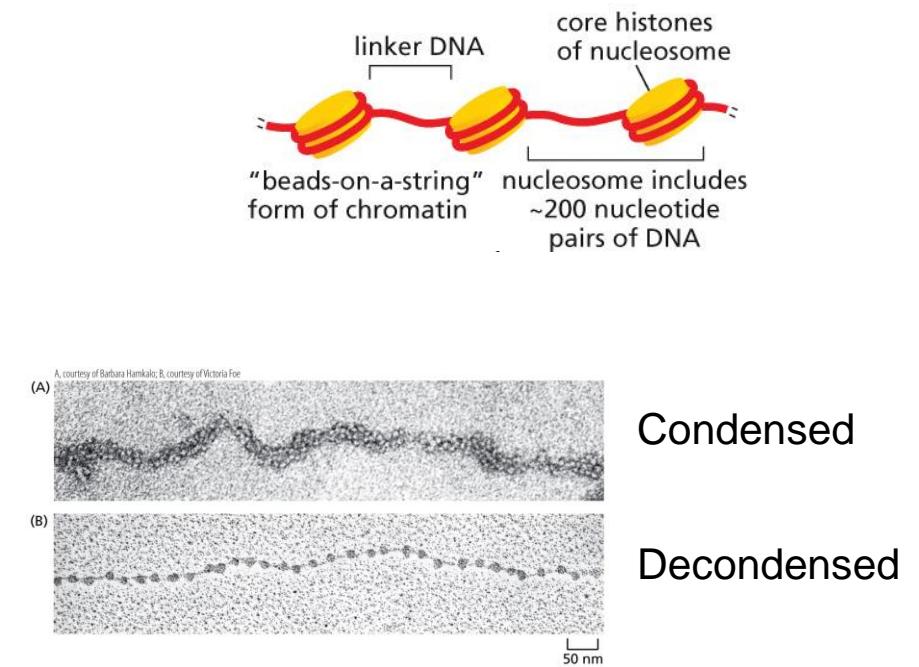
Chromosomes: The DNA space saver

- The DNA in our cells is compacted into tightly wound coils and loops within **chromosomes** to allow 3 billion base pairs to fit into a width of *just 2 μm*
 - Prokaryotic cells have a circular DNA molecule that condenses into a singular “chromosome”
 - Human cells have 46 linear chromosomes (23 pairs – each has a maternal and paternal version of the chromosome = homologous)

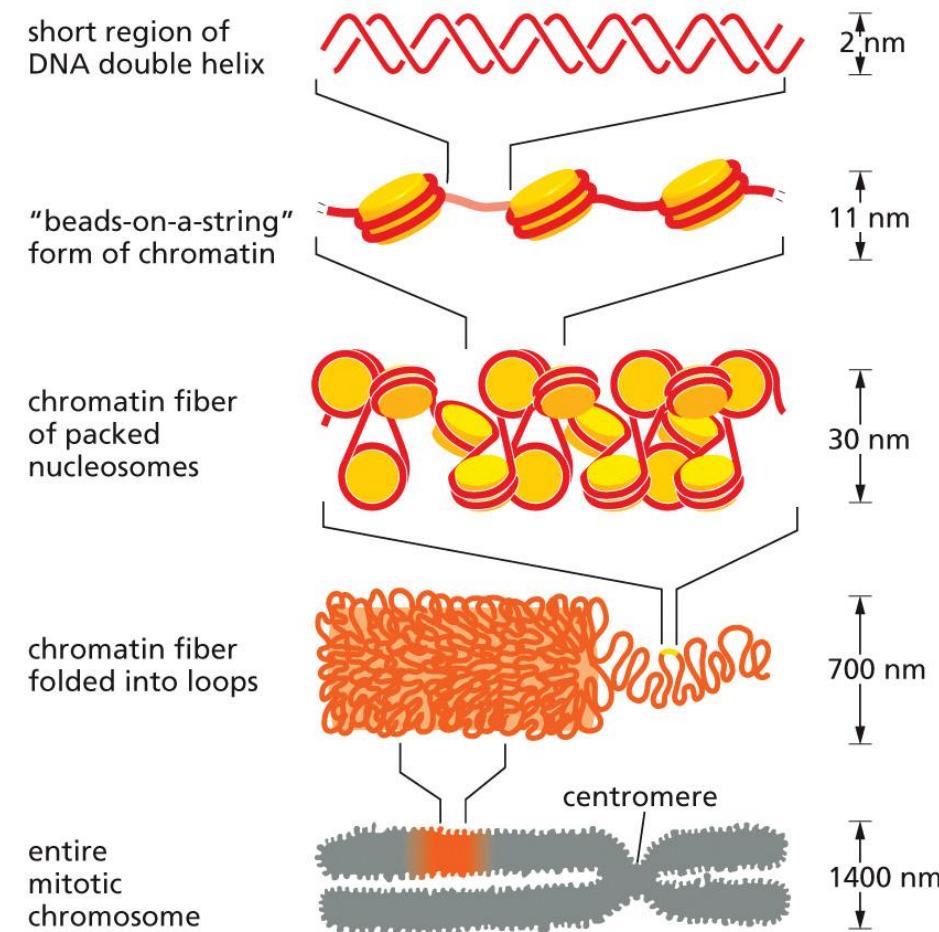


Nucleosomes are the basic units of eukaryotic chromosome structure

- DNA wraps around proteins to form *nucleosomes* creating a “*bead on a string*” appearance
- Each nucleosome is composed of DNA wrapped around a core of eight **histone** proteins
- Positively charged amino acids in each histone bind tightly to the negatively charged backbone of DNA
- “Tails” on each histone allow chemical modifications to regulate how accessible sections of chromosomes are for transcription

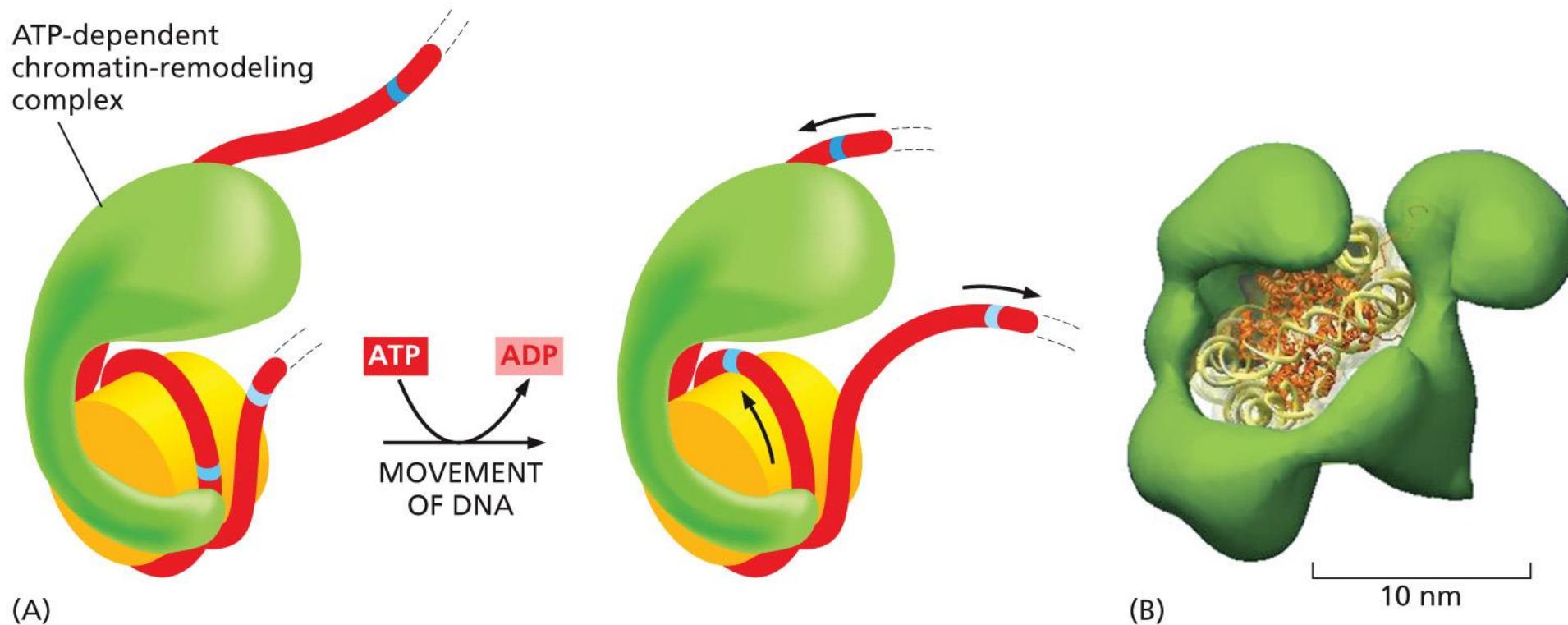


DNA in chromosomes is highly condensed into chromatin



NET RESULT: EACH DNA MOLECULE HAS BEEN
PACKAGED INTO A MITOTIC CHROMOSOME THAT
IS 10,000-FOLD SHORTER THAN ITS FULLY
EXTENDED LENGTH

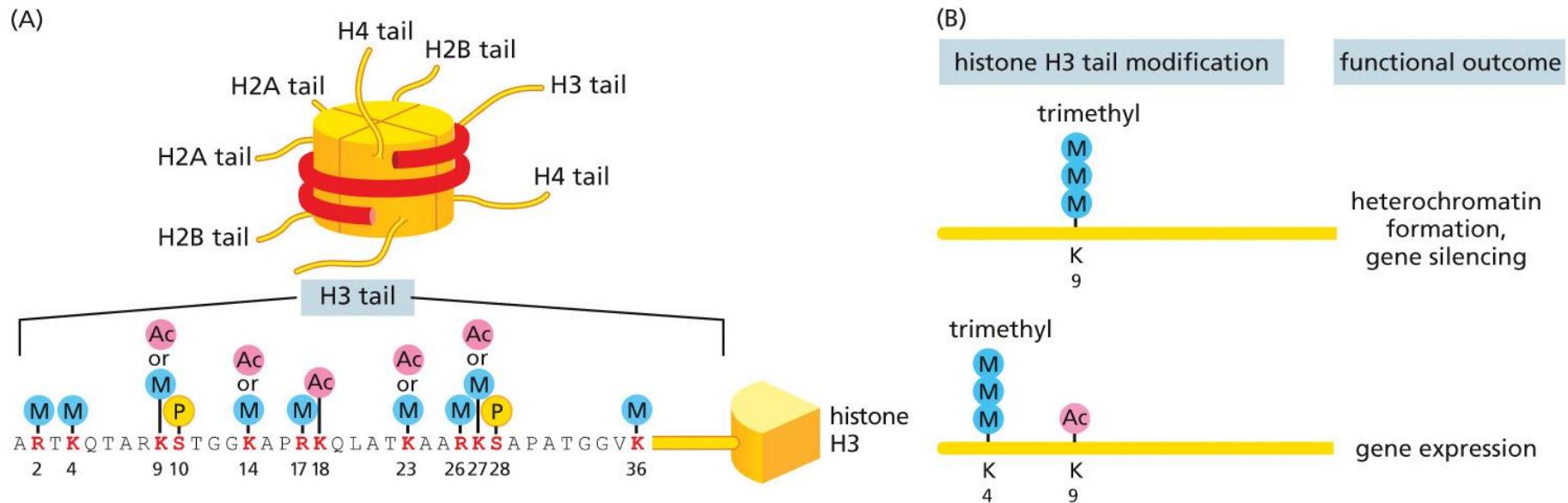
Changes in nucleosome structure allow access to DNA: *chromatin-remodeling complexes*



B adapted from A.E. Leschziner et al., *Proc. Natl. Acad. Sci. USA* 104:4913–4918, 2007

Chromosome remodeling complexes can expose or conceal regions of DNA around a histone (ATP driven movement of DNA strand)

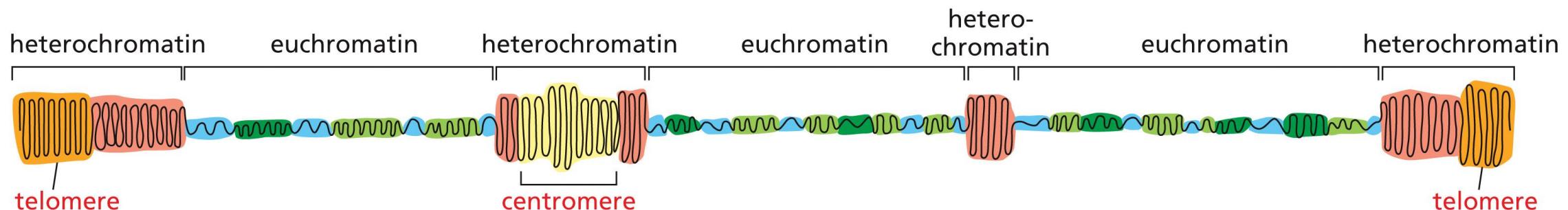
Changes in nucleosome structure allow access to DNA: histone modifying enzymes



- Histones have tails that can be modified through reversible attachment of **methyl, acetyl, phosphate, or other groups**
 - Generally, methylation leads to gene silencing and acetylation leads to gene expression, but it's the *combination of modifications* that ultimately dictates gene expression

Chromosomes have a mix of highly condensed (heterochromatin) and extended chromatin (euchromatin)

- Chromatin is not uniformly packed and patterns can be different from cell type to cell type
 - Most cells only express half of their genes at any given time
- Depending on type of chromatin modification regions can be defined as:
 - **Euchromatin:** active/open or less condensed, gene-rich and more easily transcribed
 - Generally due to acetylation of histones
 - **Heterochromatin:** inactive/closed or condensed, gene-poor, transcriptionally silent
 - Generally due to methylation of histones



Squarecap #3

Learning Objectives for Chapter 5 & 6:

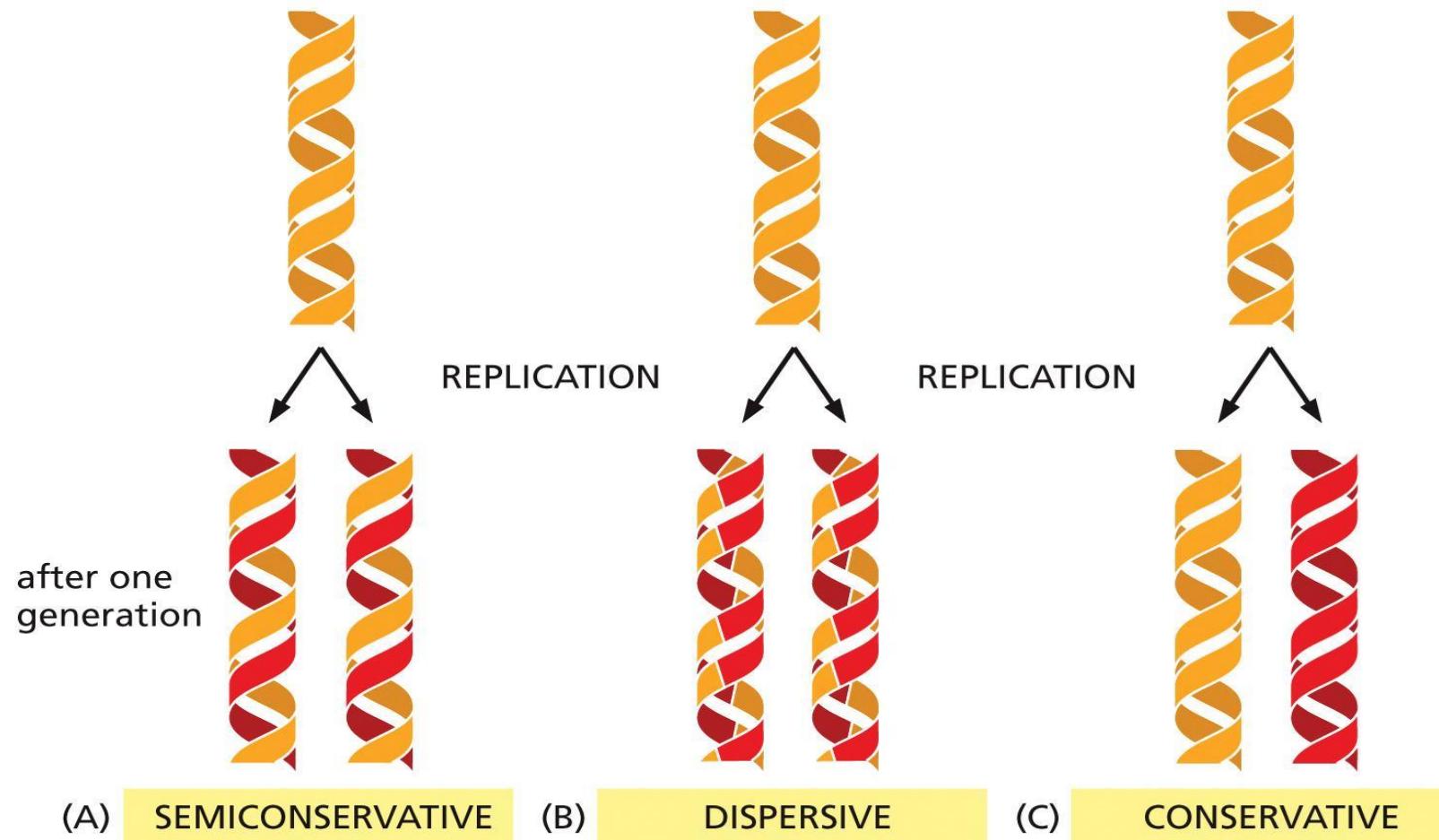
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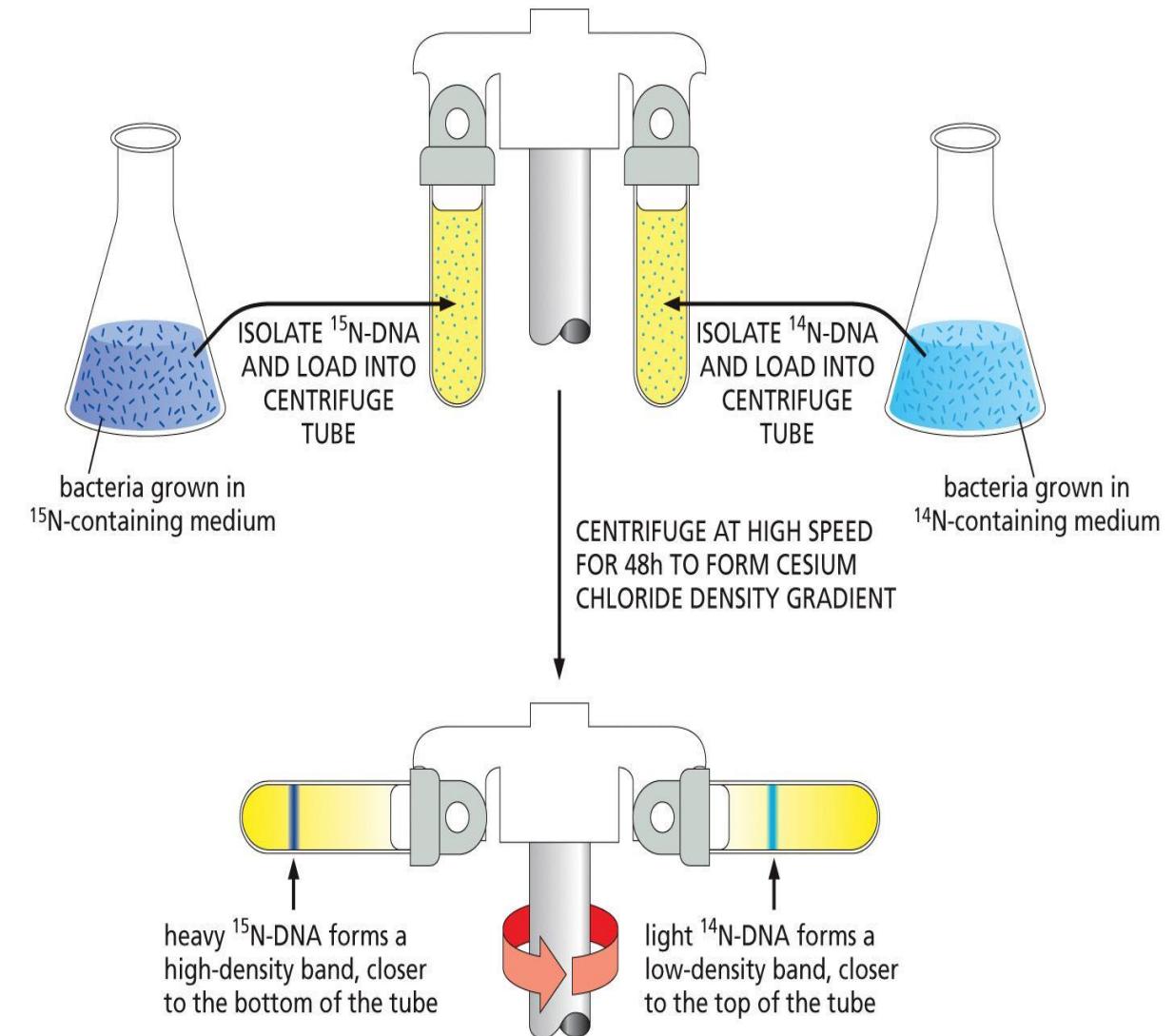
Three possible models of DNA replication



How do we know which method of DNA replication is correct? Meselson–Stahl Experiment

Experimental setup:

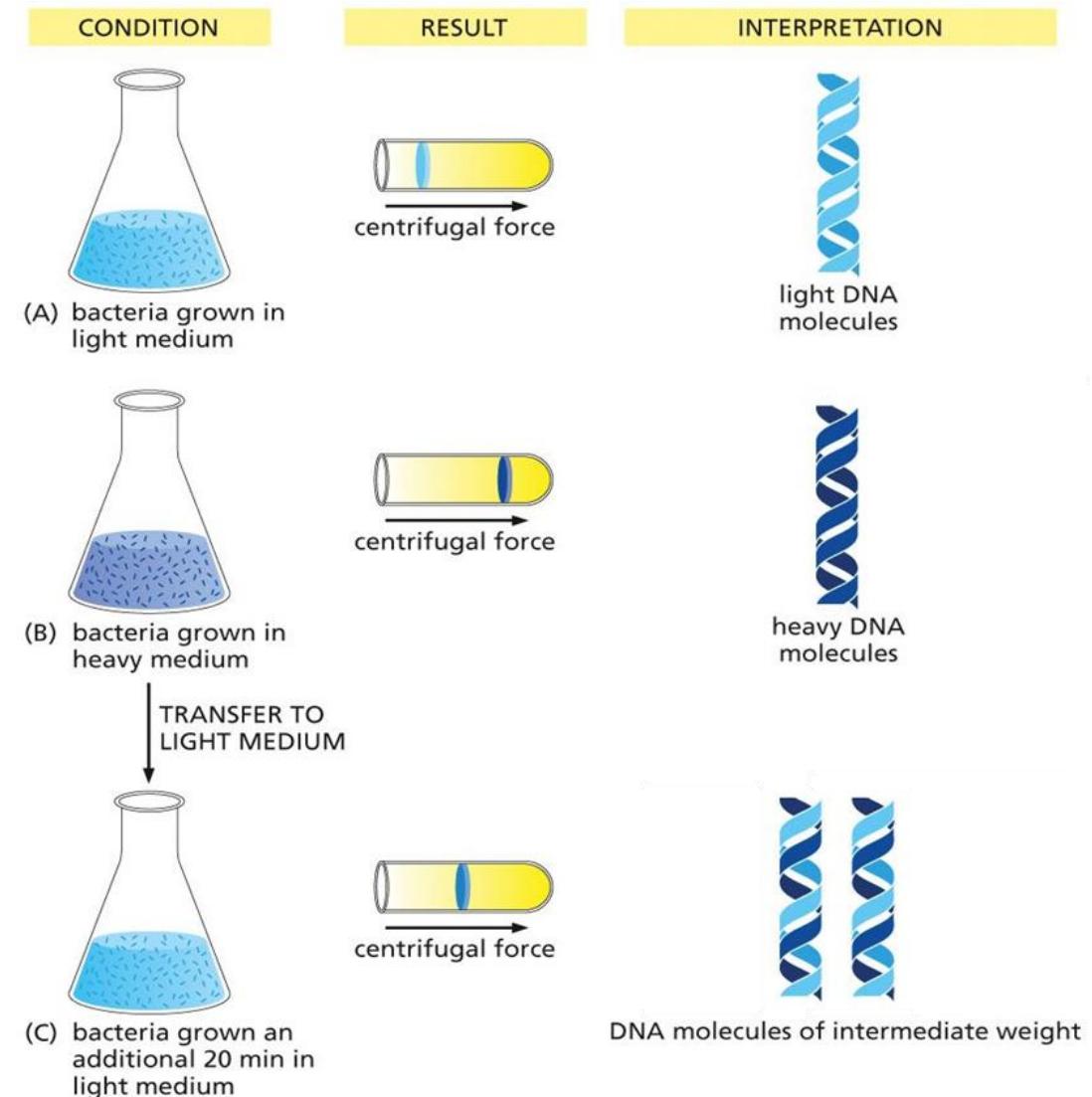
- Bacteria were grown with either ^{15}N (the heavy isotope) or ^{14}N (the light isotope) to incorporate into the bacteria's DNA
- DNA was then isolated and centrifuged within a density gradient solution to form a distinct band at a density that matches the salt surrounding it.



Meselson–Stahl Experiment Demonstrated Semiconservative DNA Replication

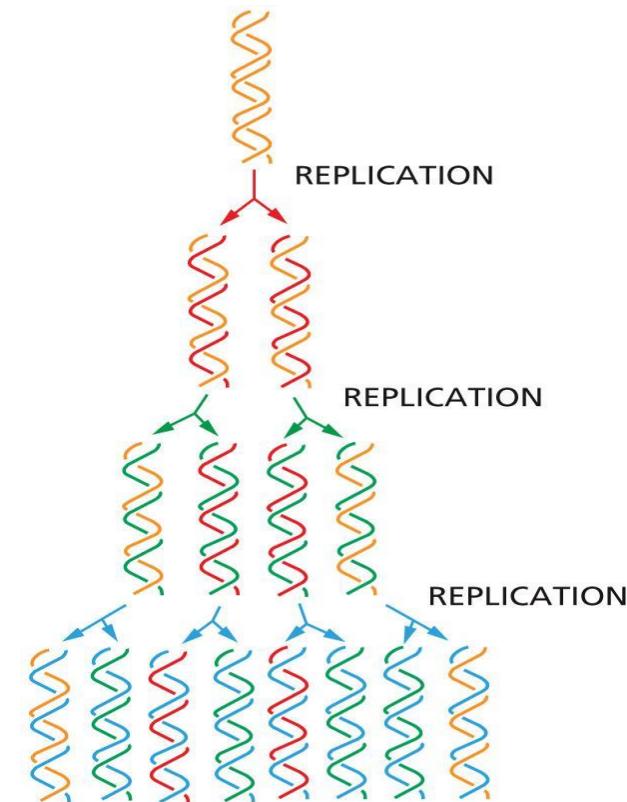
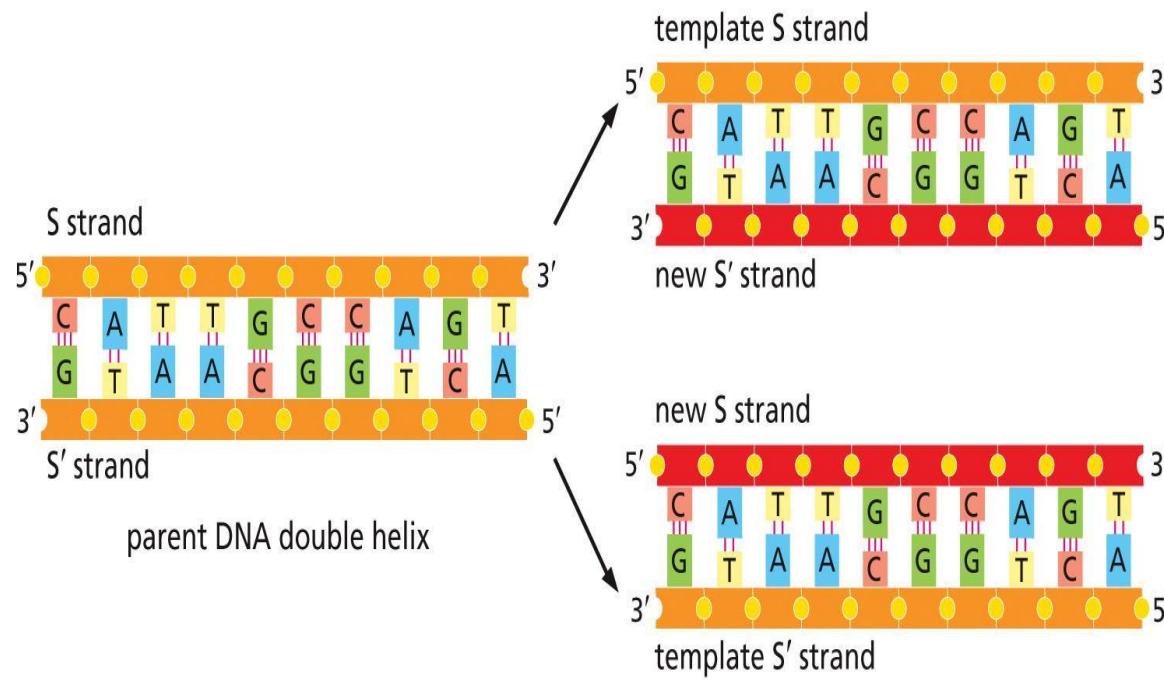
After allowing bacteria with “heavy” DNA to grow in “light” media:

- Newly synthesized DNA banded at a density that was halfway between light and heavy
- Automatically ruled out conservative model (as would see 2 bands, light and heavy) but could be either semiconservative or dispersive
- Application of heat to separate the strands before centrifuging led to both light and heavy bands, rather than some intermediate band sizes, confirming **semiconservative replication**

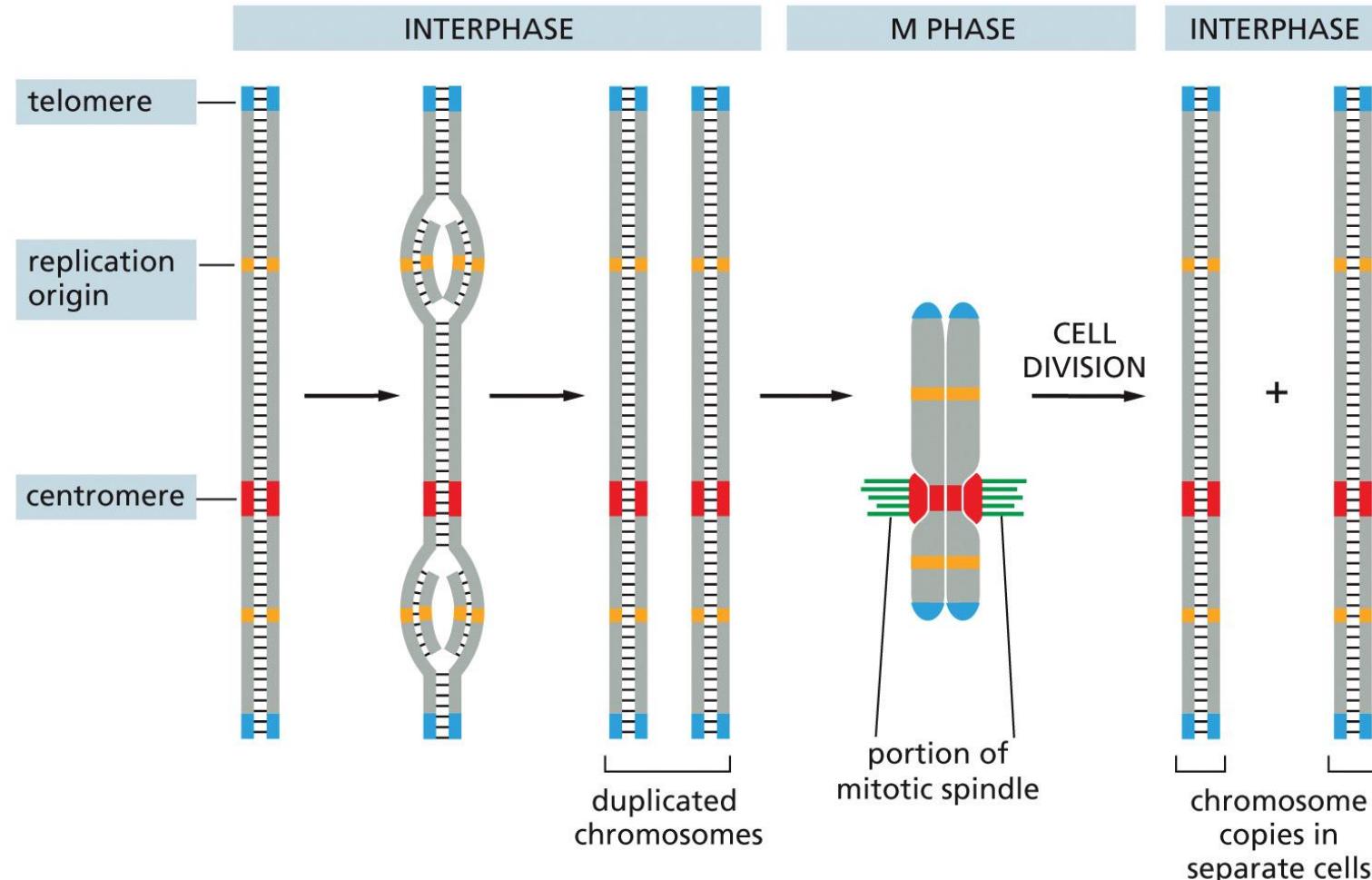


DNA serves as a template for its own replication

- DNA replication is **semiconservative**
 - Each daughter DNA double helix is composed of one conserved (old) strand and one newly synthesized strand.

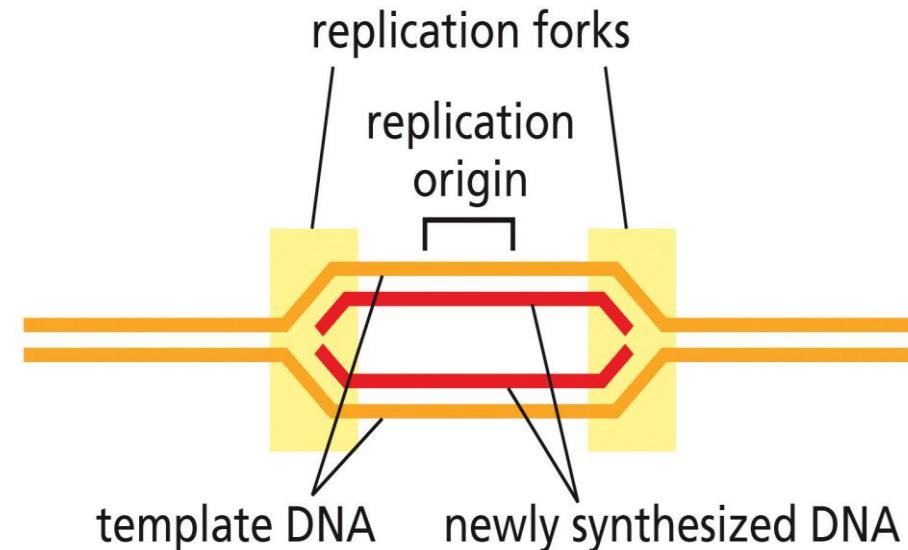
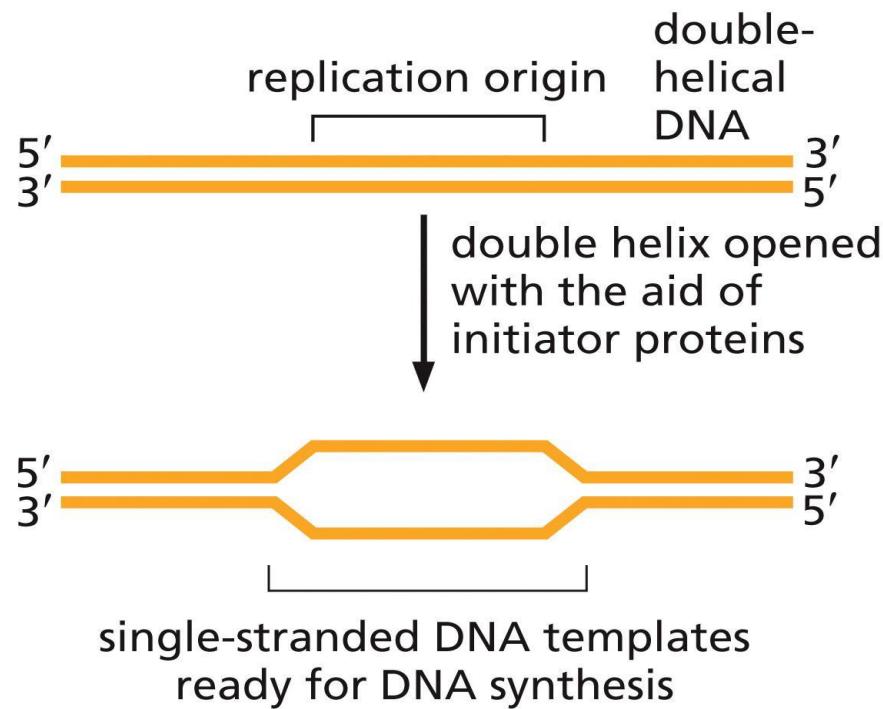


3 essential DNA sequence elements needed for chromosomal replication



1. **Telomere:** repeated nucleotide sequence of DNA ends of chromosomes to prevent shortening upon multiple replications
2. **Replications of origin:** necessary to have DNA synthesis occur via polymerase protein complexes
3. **Centromere:** repetitive nucleotide region that holds duplicated chromosomes together and permits proper segregation during mitosis (cell division)

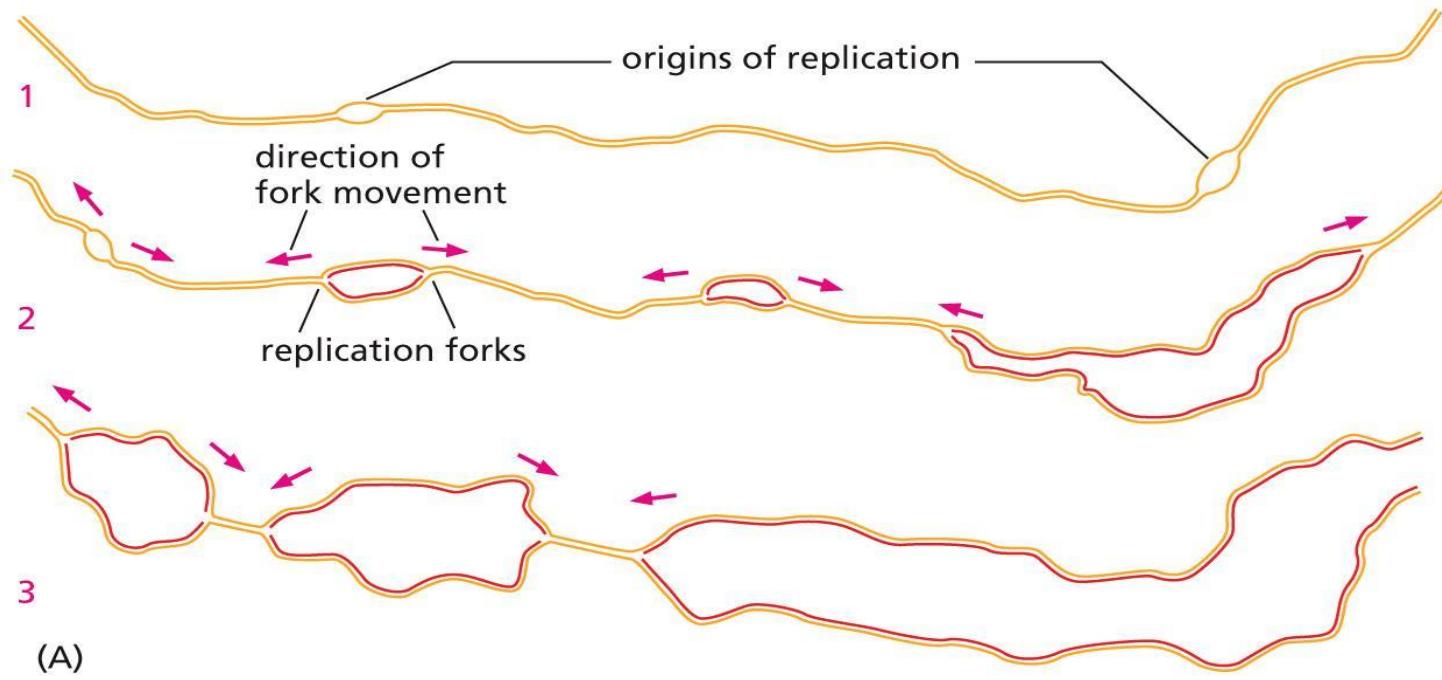
DNA synthesis begins at replication origins



- Replication origins are short segments of DNA, with a high concentration of AT nucleotides and are unzipped by initiator proteins to create a replication fork
- Replication forks form in each direction and additional proteins move along the strands synthesizing new DNA

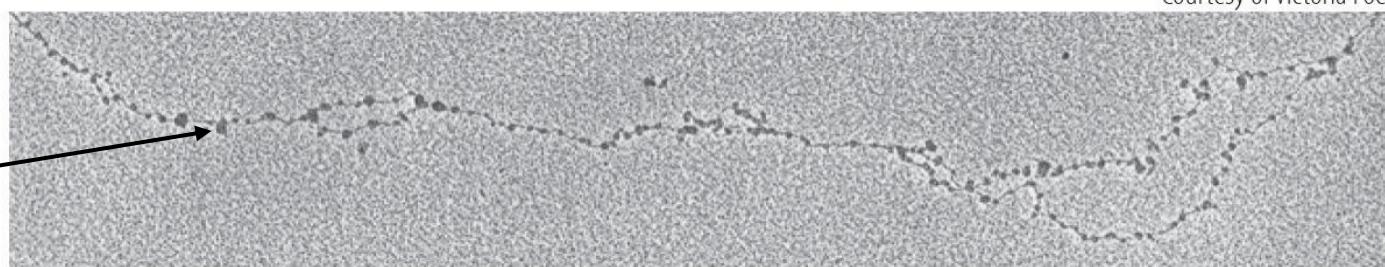
DNA Replication is bidirectional

- Replication occurs at multiple spots across linear chromosomes
- **Replication bubbles** form due to bidirectional DNA replication at each origin of replication



Courtesy of Victoria Foe

nucleosomes



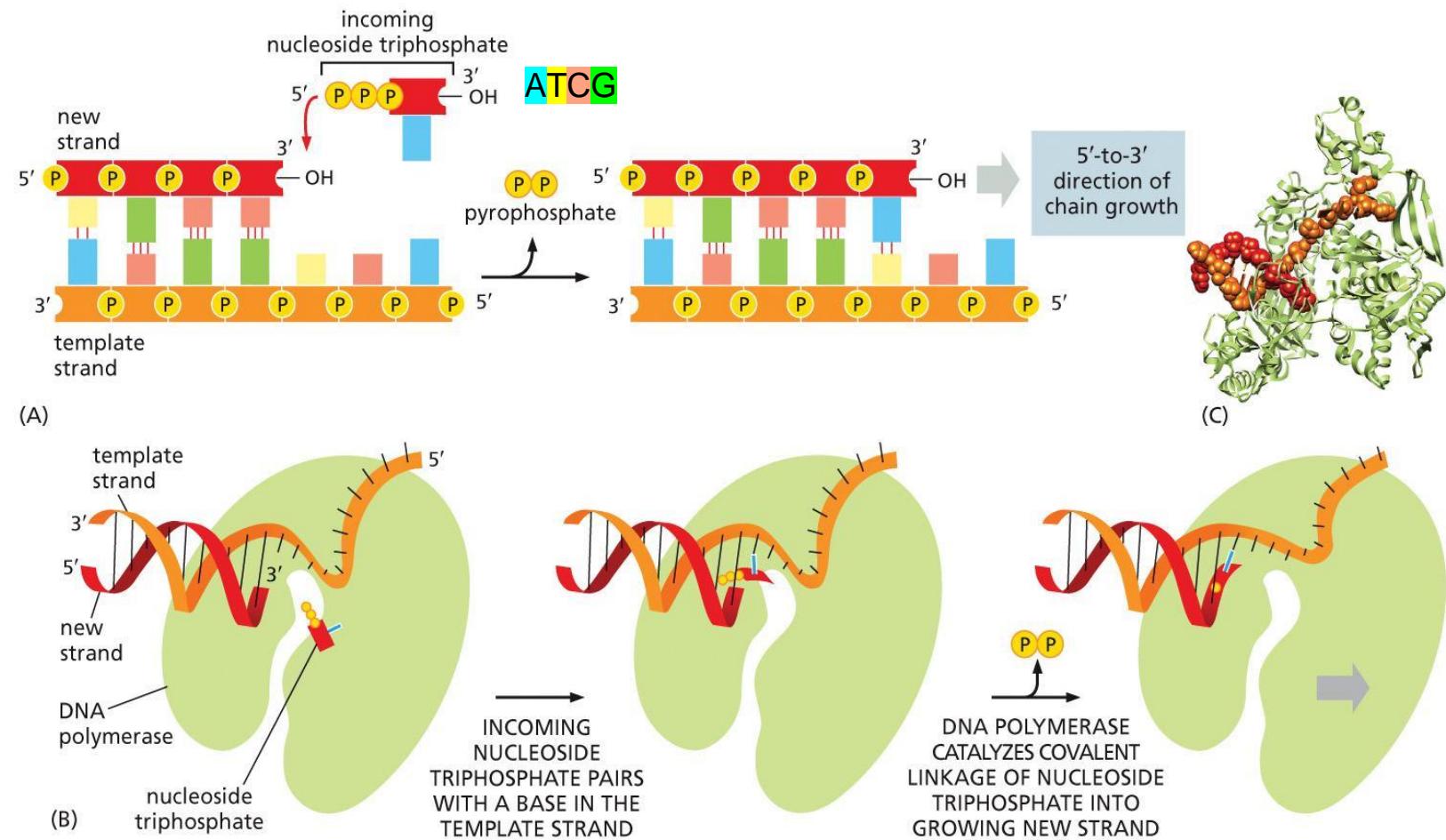
(B)

Electron micrograph of DNA replication in an early fly embryo

$0.1 \mu\text{m}$

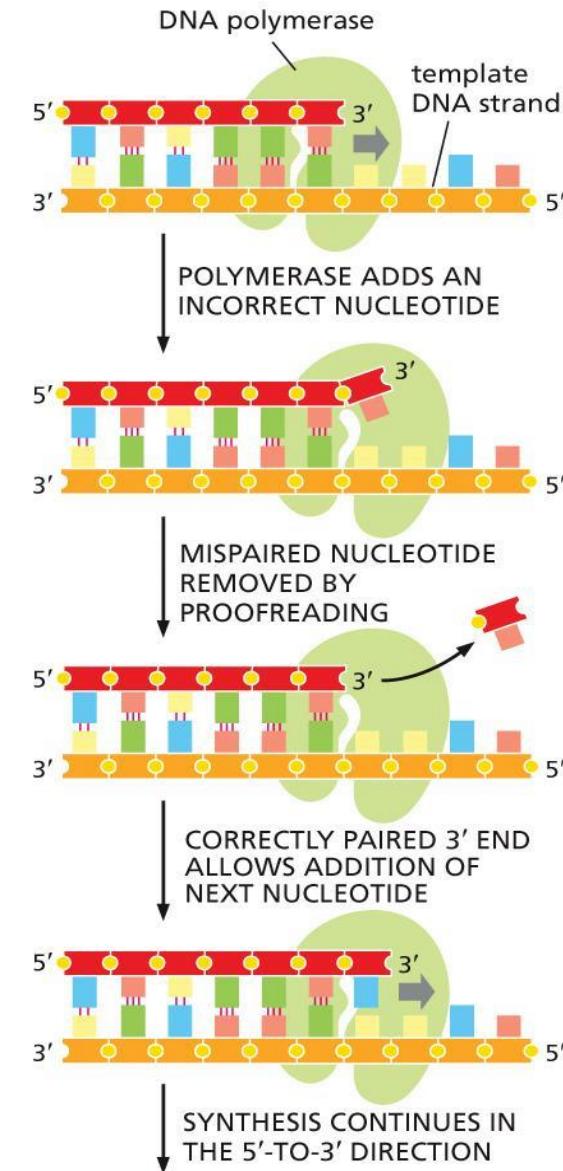
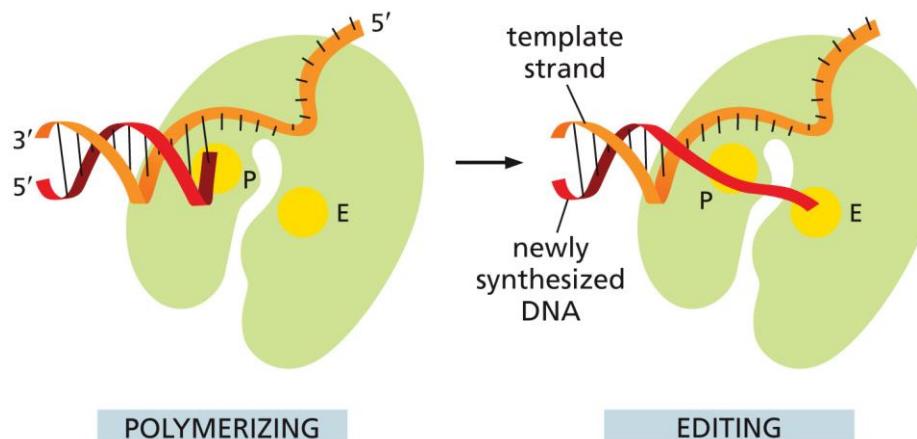
DNA polymerase synthesizes DNA using parental strand as a template

- A template and a base-paired 3'-OH is needed for **DNA polymerase** to synthesize new DNA strand in the 5'-to-3' direction
- Incoming **deoxyribonucleoside triphosphate** base pairs with the nucleotide in the template strand and forms a phosphodiester bond
- Energy provided by hydrolysis of the high energy phosphate bond of the incoming nucleoside triphosphate (like ATP)



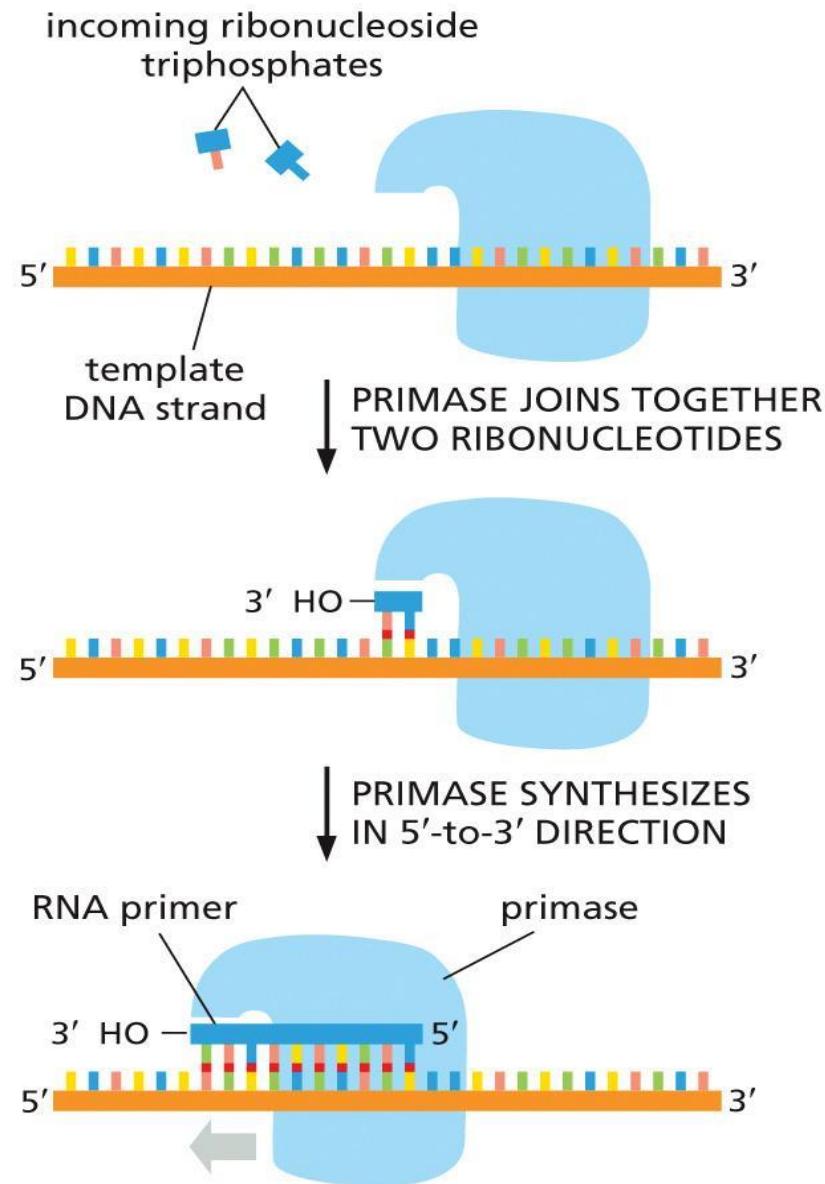
DNA polymerase proofreads and corrects

- 1/100,000 nucleotides added are incorrect! These mistakes could be catastrophic without proofreading
- DNA polymerases are self-correcting:
 - Before adding a new nucleotide, it checks that the previously added nucleotide is correct via $3' \rightarrow 5'$ **exonuclease activity** to cleave incorrect nucleotide
 - Polymerization and proofreading are carried out by two different catalytic domains on the enzyme



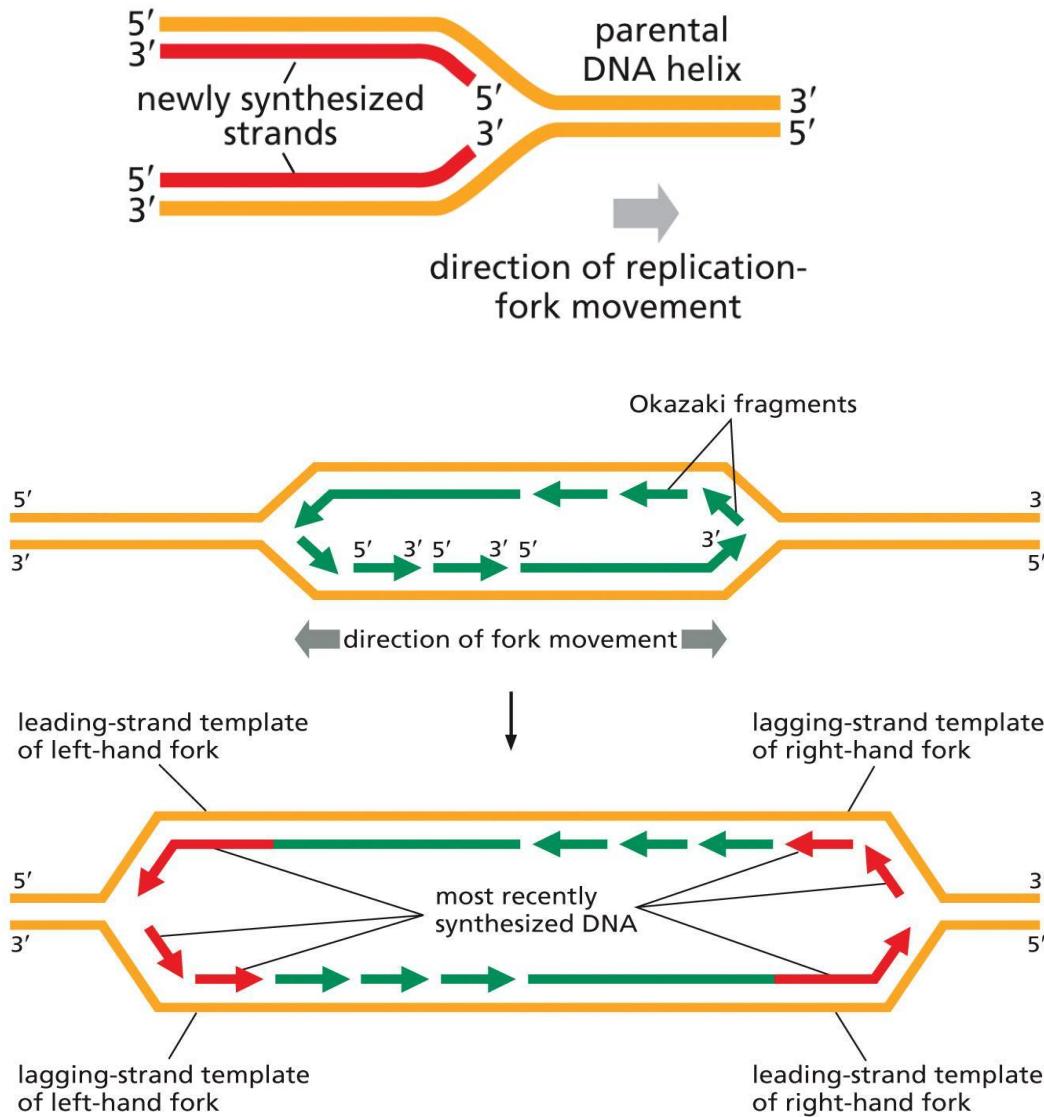
Short segment of RNA is required to initiate DNA synthesis

- Primase adds a 10-20 nucleotide long *RNA primer* in the 5'-to-3' direction prior to DNA nucleotide polymerization
- Unlike DNA polymerase, primase does not require a 3' OH to extend polymerization, and so it can begin the process then allow DNA polymerase to take over



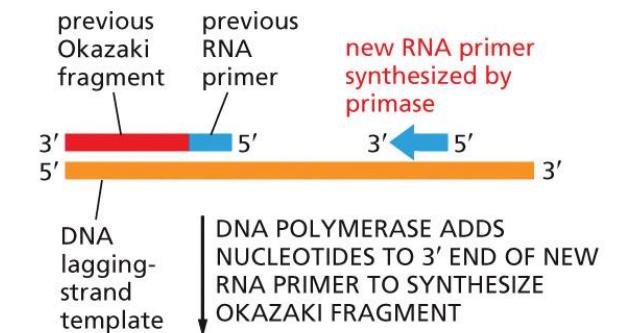
At each replication fork there is a leading and a lagging strand

- As DNA polymerases need a base-paired 3'-OH to add on to, synthesis must occur in the 5'-to-3' direction
- Strands are antiparallel so templates are running in opposite directions
- Leading strand** runs 3'-to-5' and is synthesized continuously
- Lagging strand** runs 5'-to-3' and is made discontinuously as a series of short DNA segments (Okazaki fragments)



Multiple enzymes are required to synthesize the lagging strand

- RNA primers (~10-20 bp long) are laid down by **primase** at multiple intervals (~every 200 bp)
- DNA polymerase** (DNA pol III) extends the primers producing Okazaki fragments
- Nucleases** degrade the RNA in the RNA-DNA hybrid
- Repair polymerases** (DNA pol I) replaces RNA primer with DNA
- DNA ligase** uses a molecule of ATP to catalyze a phosphodiester bond between the 5'-phosphate of one fragment and the 3'-OH end of the next to join the two DNA ends together



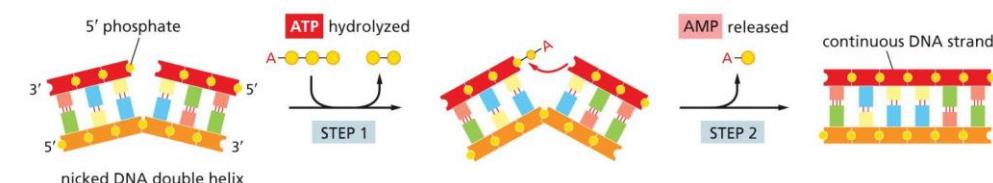
DNA POLYMERASE FINISHES OKAZAKI FRAGMENT



PREVIOUS RNA PRIMER REMOVED BY NUCLEASES AND REPLACED WITH DNA BY REPAIR POLYMERASE



NICK SEALED BY DNA LIGASE



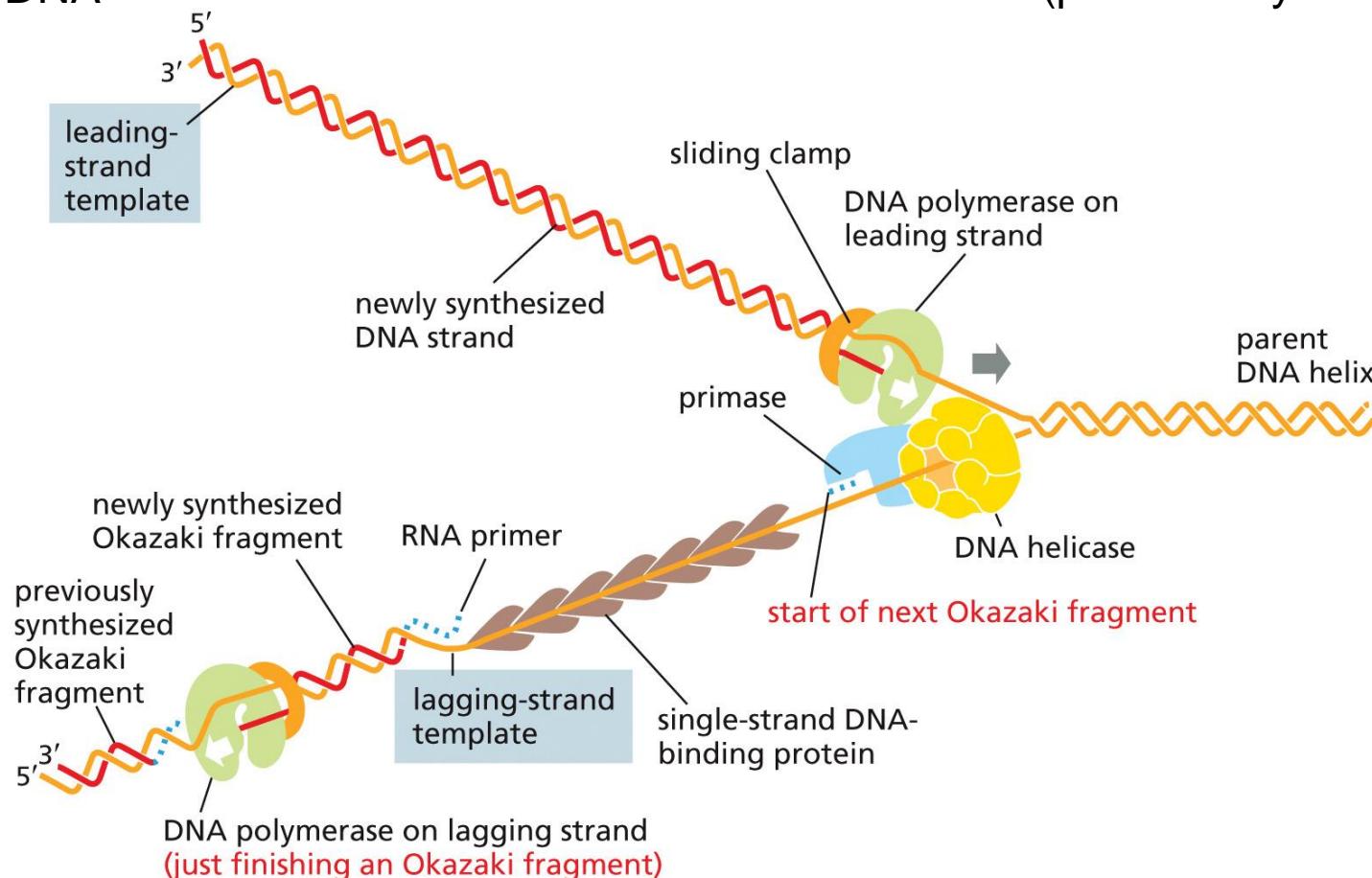
Proteins at a replication fork cooperate to form a replication machine

Sliding Clamp Protein (orange)

binds to DNA polymerase and prevents it from falling off the DNA (stabilization)

Helicase (yellow)

Recruited by initiator proteins, unravel double-stranded DNA at the replication fork (powered by ATP)

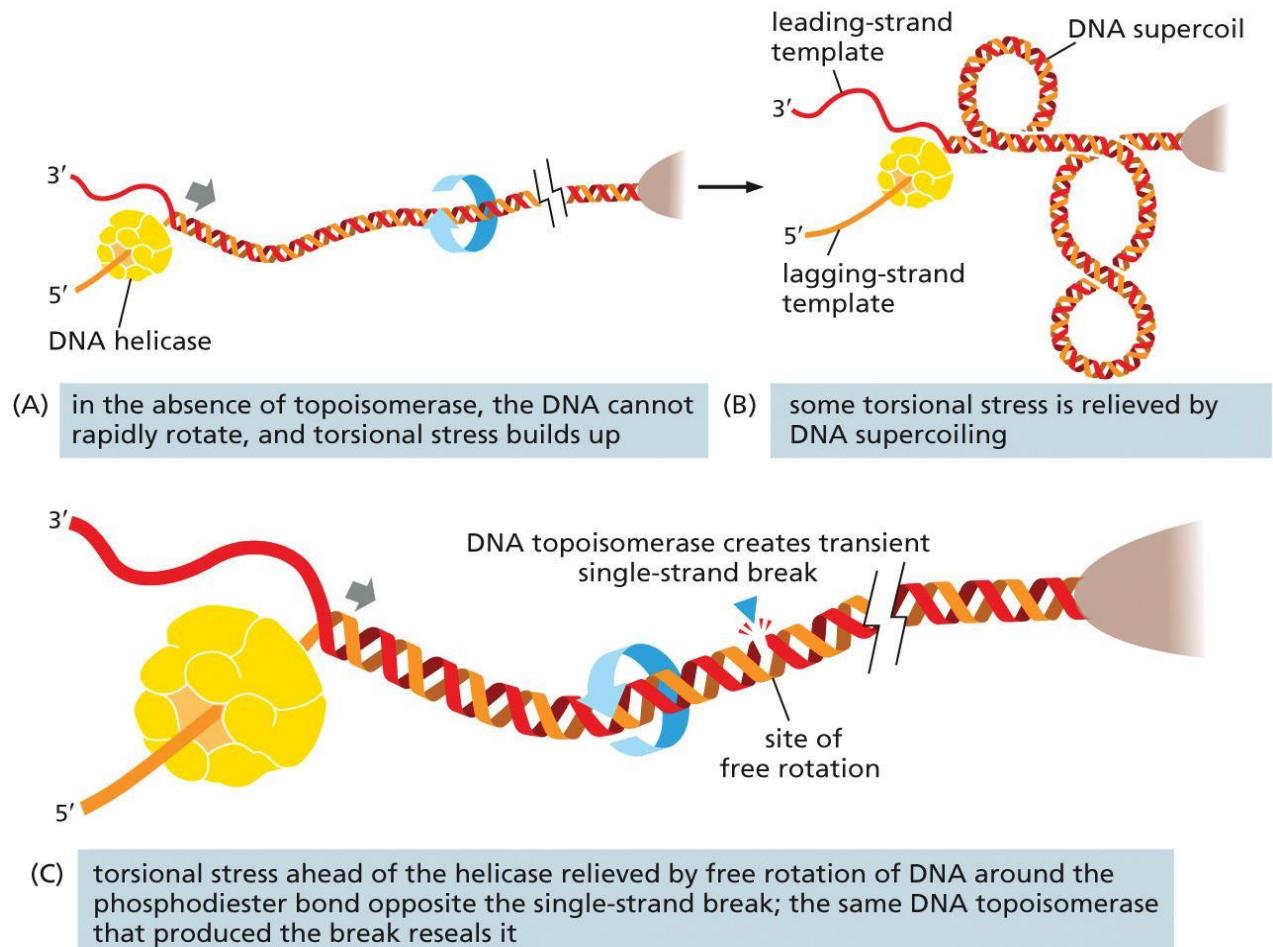


Single-stranded binding proteins (SSBPs) (brown) keep single-stranded DNA unwound during replication

See “Replication I and II” videos in Ch6 Videos

Topoisomerases relieve tension that builds in front of the replication fork

- As helicase moves forward unwinding double helix, the DNA ahead of the fork gets wound more tightly and would make unwinding more difficult
- Topoisomerases create nicks in the DNA strand to temporarily relieve the tension and then reseals the nick



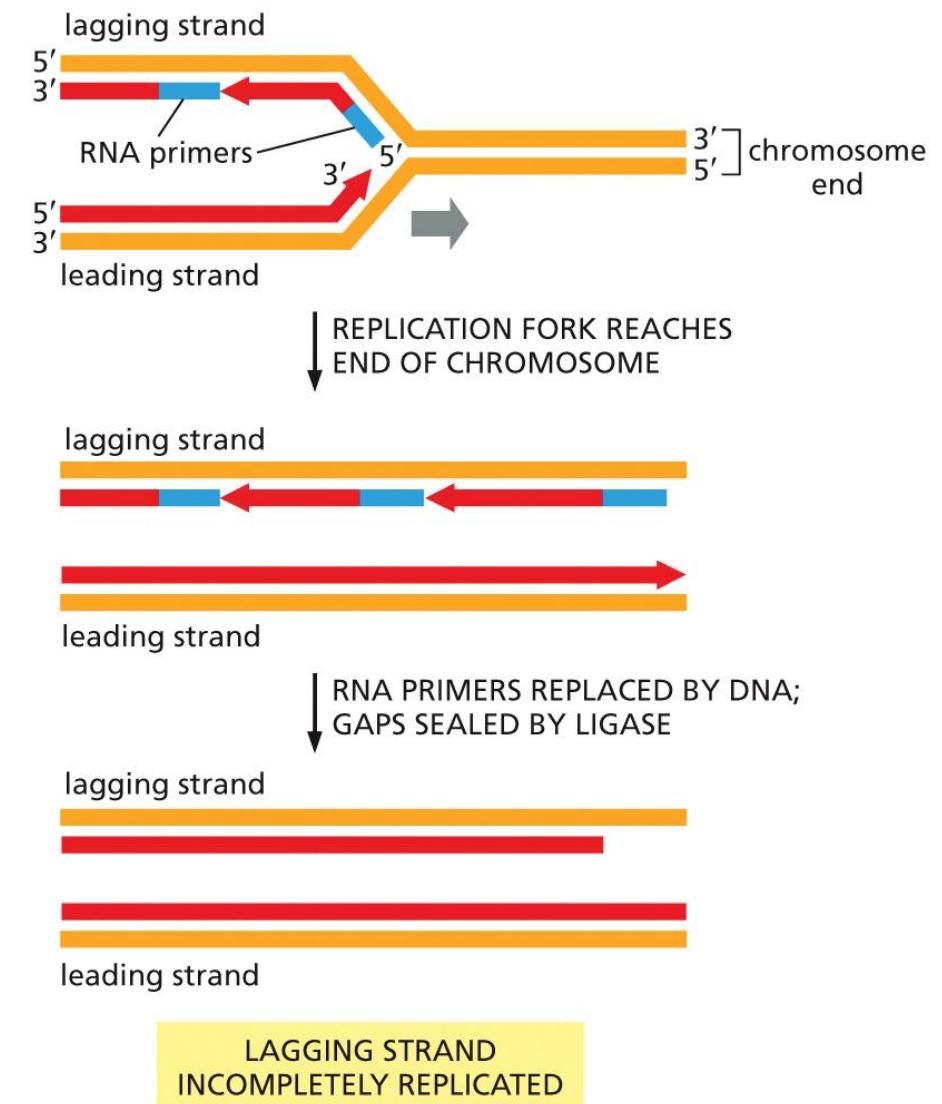
Summary proteins involved in DNA replication

TABLE 6–1 PROTEINS INVOLVED IN DNA REPLICATION

Protein	Activity
DNA polymerase	catalyzes the addition of nucleotides to the 3' end of a growing strand of DNA using a parental DNA strand as a template
DNA helicase	uses the energy of ATP hydrolysis to unwind the DNA double helix ahead of the replication fork
Single-strand DNA-binding protein	binds to single-stranded DNA exposed by DNA helicase, preventing base pairs from re-forming before the lagging strand can be replicated
DNA topoisomerase	produces transient nicks in the DNA backbone to relieve the tension built up by the unwinding of DNA ahead of the DNA helicase
Sliding clamp	keeps DNA polymerase attached to the template, allowing the enzyme to move along without falling off as it synthesizes new DNA
Clamp loader	uses the energy of ATP hydrolysis to lock the sliding clamp onto DNA
Primase	synthesizes RNA primers along the lagging-strand template
DNA ligase	uses the energy of ATP hydrolysis to join Okazaki fragments made on the lagging-strand template

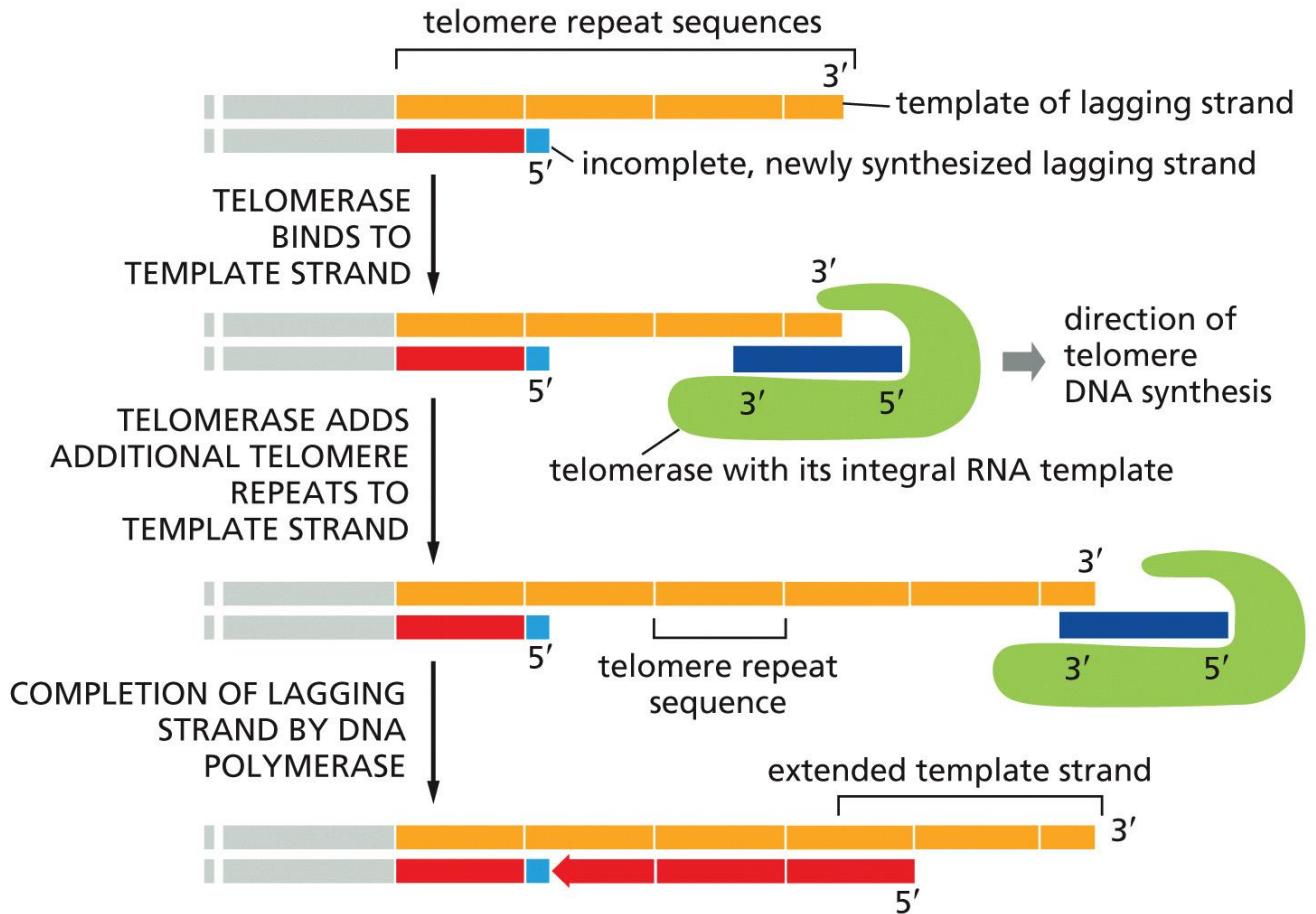
Telomerase replicates the ends of eukaryotic chromosomes

- Because synthesis occurs only in the 5'-to-3' direction, the lagging strand of the replication fork is synthesized in discontinuous fragments, eventually leading to the “**end-replication problem**: Lagging strand would become shorter with each round of replication
- Telomerase** solves this problem by adding repetitive sequences to chromosome ends



Telomerase replicates the ends of eukaryotic chromosomes

- Telomerases have an RNA template embedded into the protein for further 3' end extension of template DNA strand (the one being copied)
- This allows for complete synthesis of the lagging strand



See “Telomerase Replication” in Ch6 Videos

Most cells have a limited lifespan

- In multicellular organisms, telomerase function is restricted to germ line cells and a few other types of actively proliferating cells
- Telomere shortening occurs with each cell division in most other cells. Loss of telomeres ultimately leads to apoptosis (cell death)
- **Immortalized cell lines:** replicate indefinitely (e.g. HeLa cells derived from cancerous cervical cells) by producing telomerase



Squarecap Q#4-5

Learning Objectives for Chapter 5 & 6:

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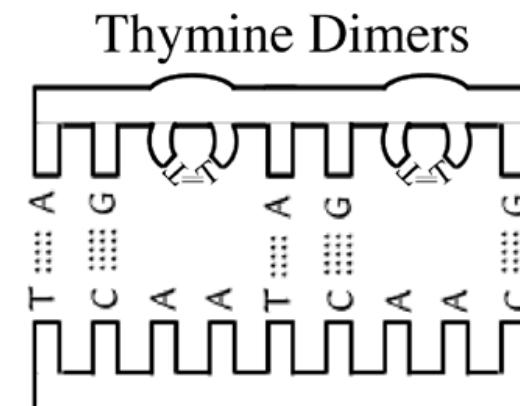
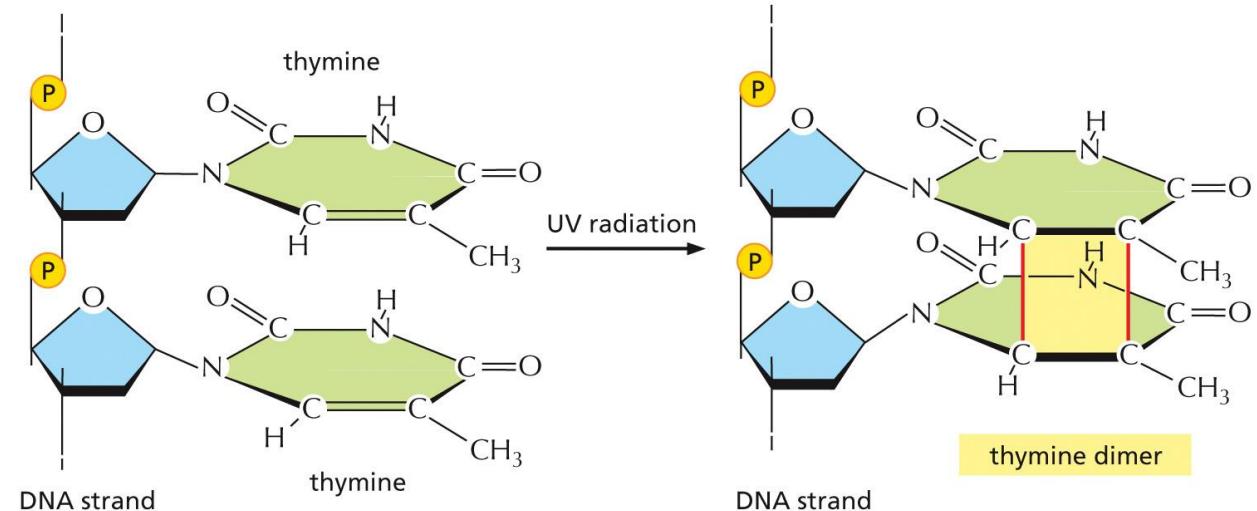
- Understand the steps of DNA replication in eukaryotic cells and describe the multiple roles of DNA polymerase and other proteins at the replication fork
- Identify causes of DNA damage in cells and describe how DNA repair mechanisms can fix damaged DNA

DNA Damage and Repair: Basic Principles

- DNA alterations, or **mutations**, can arise spontaneously during replication or through exposure to environmental (chemical or radiation) or biological (viruses) agents
- A variety of mechanisms have evolved for DNA repair and depend on how severe the damage is and whether or not the cell is undergoing division
 - A **DNA mismatch repair** system removes replication errors that escape proofreading
 - *Double-strand DNA breaks* require a different strategy for repair:
 - **Nonhomologous end joining**
 - **Homologous recombination**

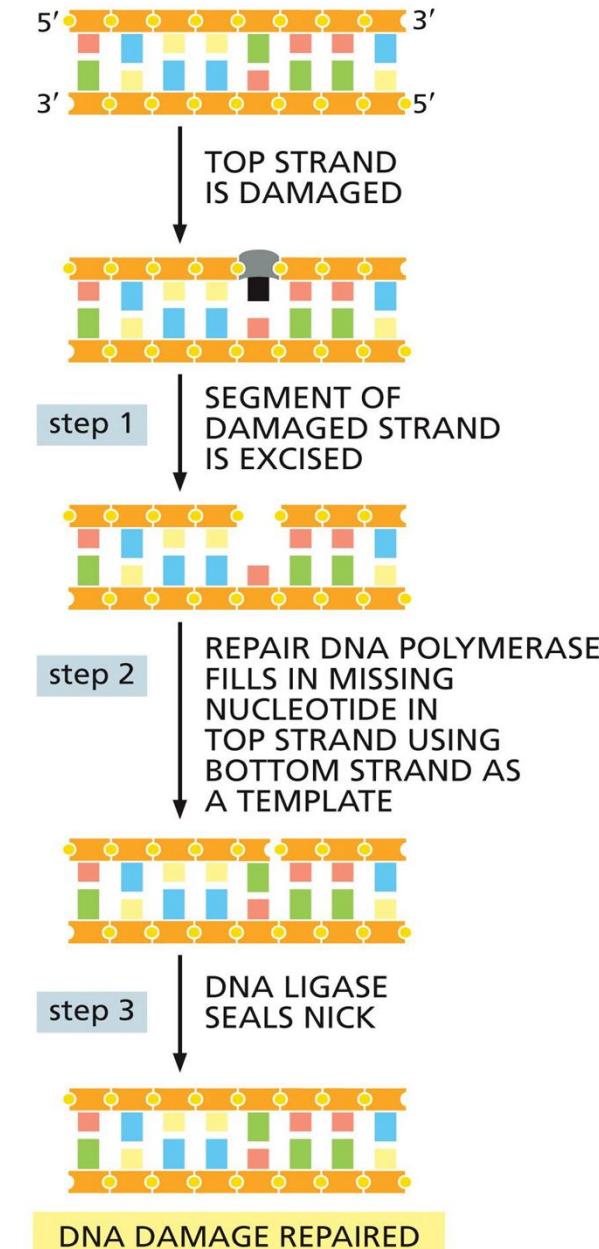
Radiation induced DNA Damage

- Ultraviolet radiation alters DNA by triggering pyrimidine dimer formation
 - Covalent bonds between adjacent pyrimidine bases
- X-rays and related types of ionizing radiation remove electrons from molecules and generate *free radicals* that can also damage DNA

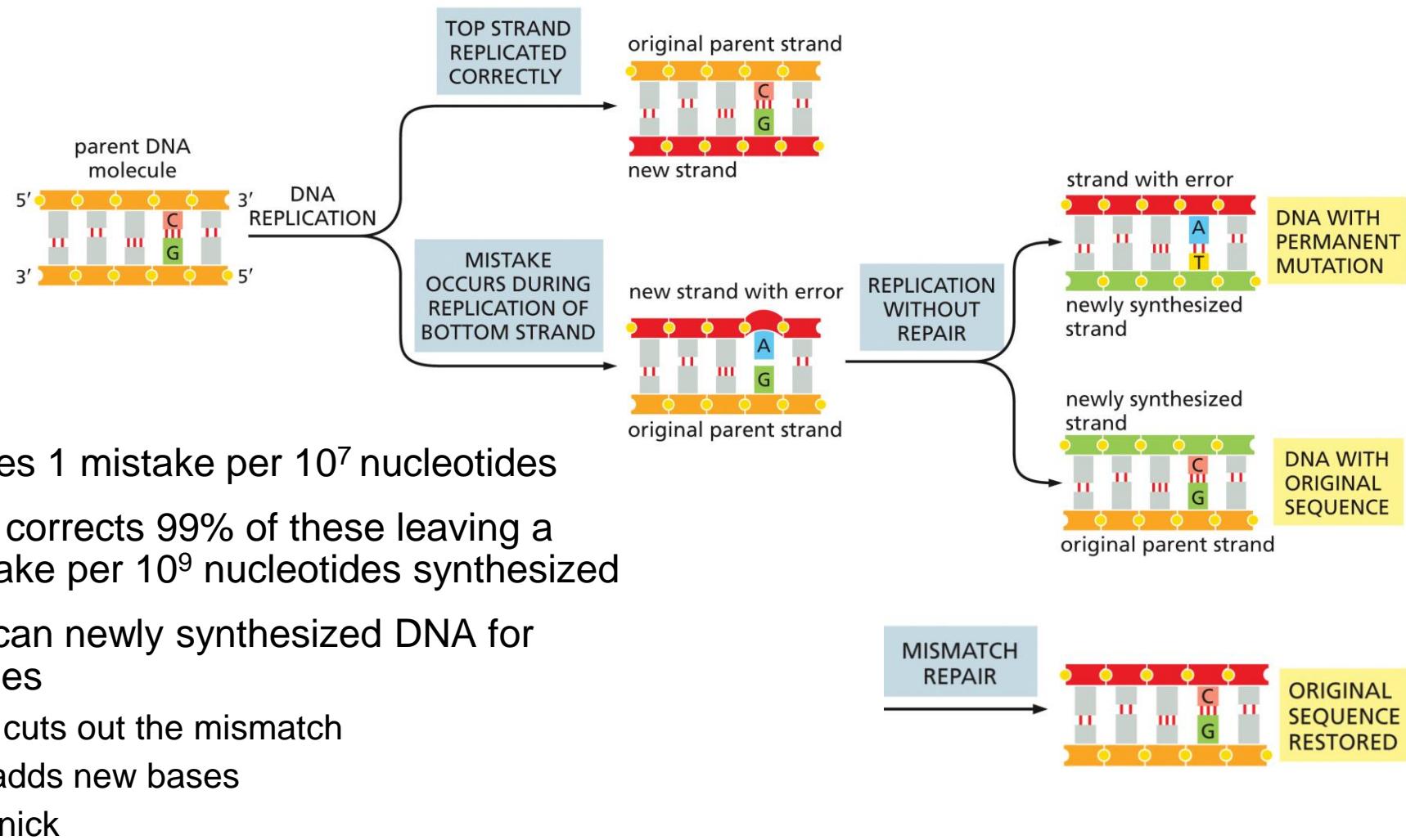


DNA repair is highly similar to lagging strand synthesis

- **Nucleases** cut the DNA strand with the damage
- **Repair DNA polymerase** fills the gaps
- **DNA ligase** restores the sugar-phosphate backbone after sealing the nick

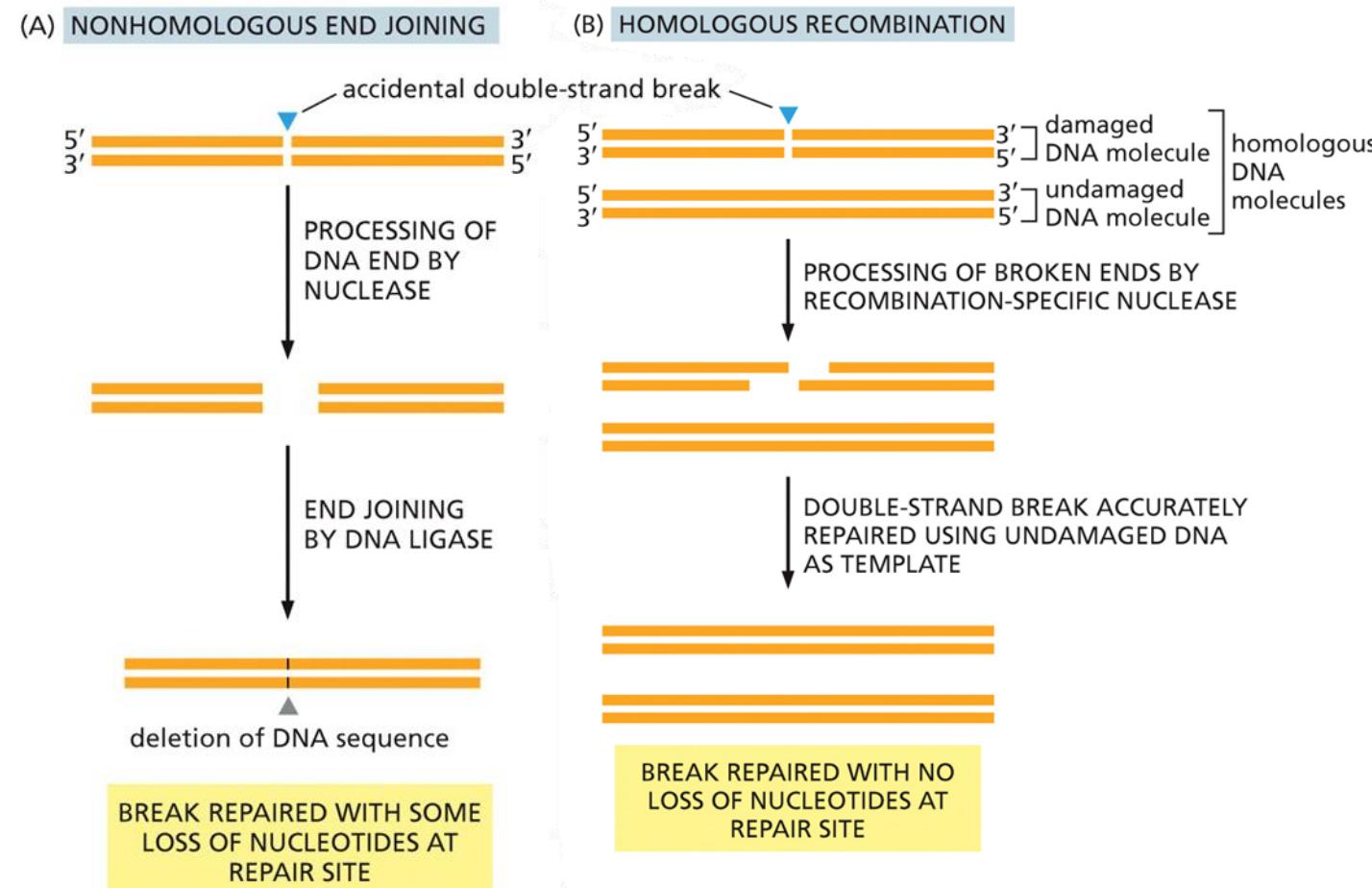


Mismatch repair (MMR) eliminates replication errors



Double strand breaks can be repaired in one of two ways

- **Nonhomologous end joining (NHEJ):** “quick and dirty” fusion of two broken ends resulting in a loss of some of the nucleotides, leading to potential mutations in the DNA sequence
- **Homologous recombination (HR):** If double strand break occurs after DNA replication has occurred, but before the chromosome copies have been separated, the undamaged double helix can be used as a template to restore original sequence



Squarecap Q#6

Learning Objectives for Chapter 5 & 6:

Ch 5:

- Identify experiments that led to the discovery of DNA as the hereditary molecule
- Understand the structure of DNA in terms of the double helix shape
- Identify how eukaryotic DNA packs into chromosomal structures and describe how chromosome structure regulates gene expression

Ch 6:

- Understand the steps of DNA replication in eukaryotic cells and describe the multiple roles of DNA polymerase and other proteins at the replication fork
- Identify causes of DNA damage in cells and describe how DNA repair mechanisms can fix damaged DNA

Feedback/Reflection

Reminder: Chapter 1-6 “Check your understanding” quizzes due by midnight tomorrow 9/18/24!

Exam next Tuesday, 9/25/24 covers Ch 1-6

