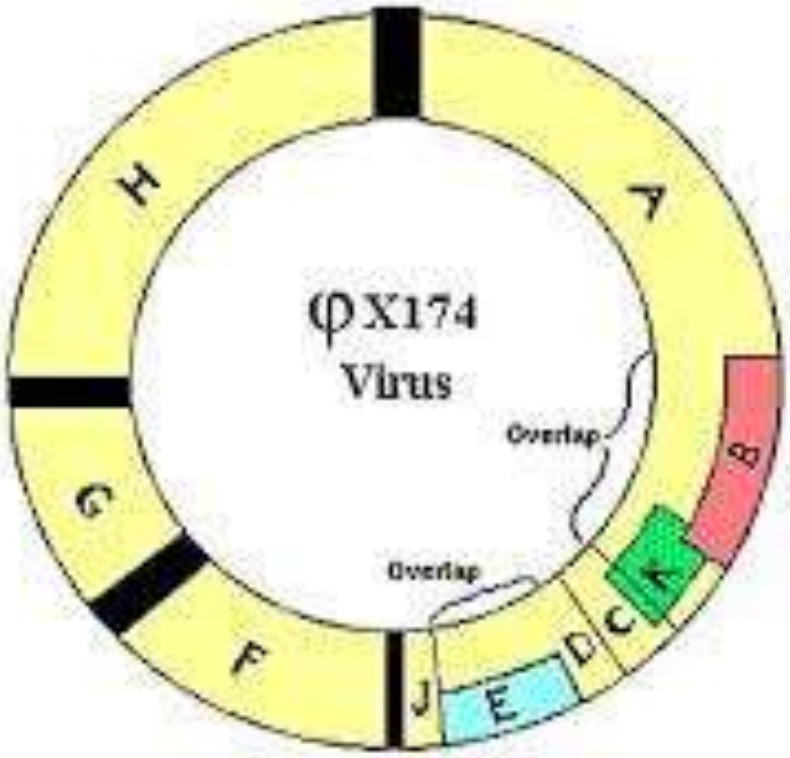
DNA Replication & Repair

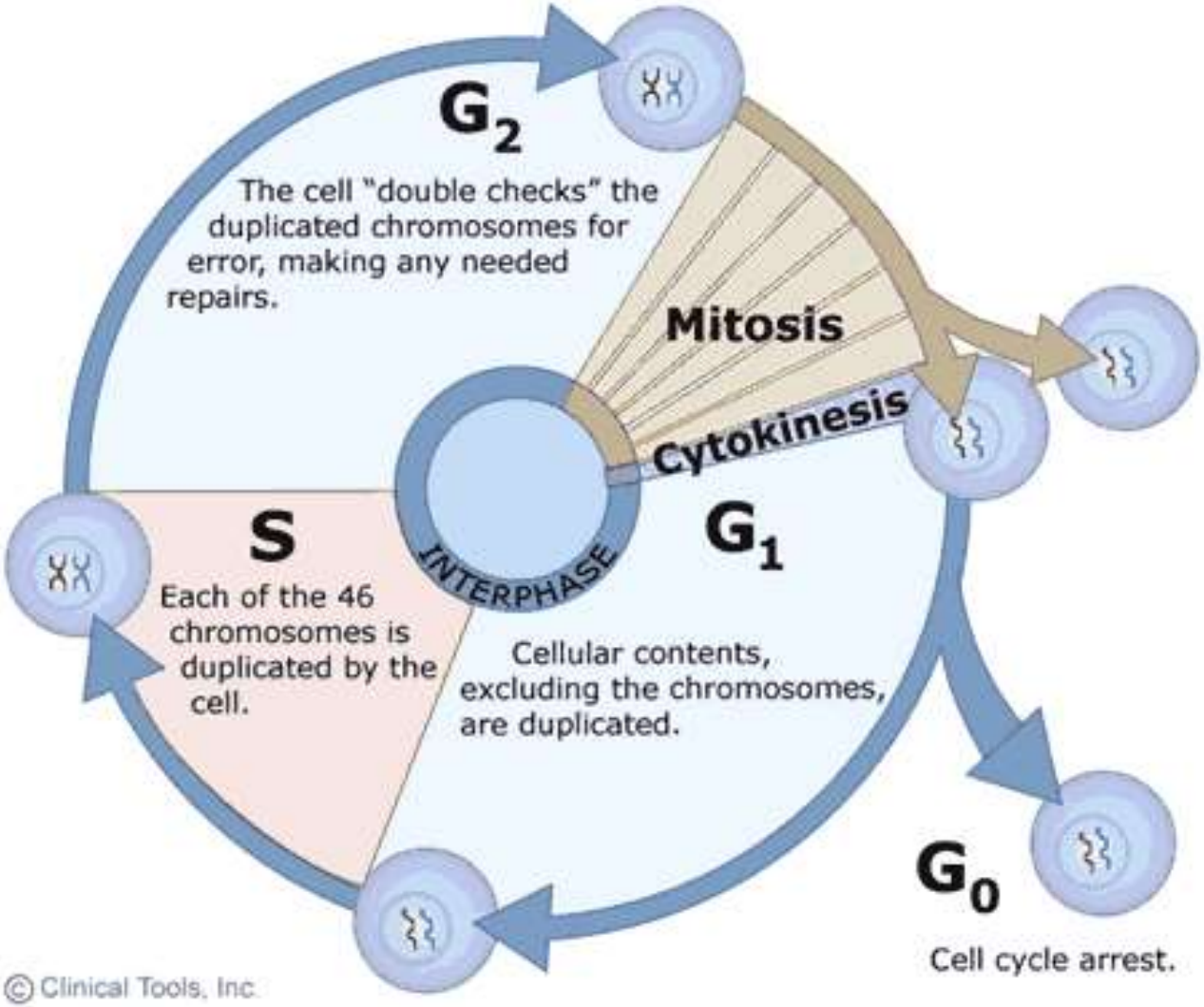
Chapter 16

Pages 292-301



Overlapping Protein Codes

Cell Cycle



http://scientopia.org/img-archive/scicurious/img_862.png

Models of DNA replication

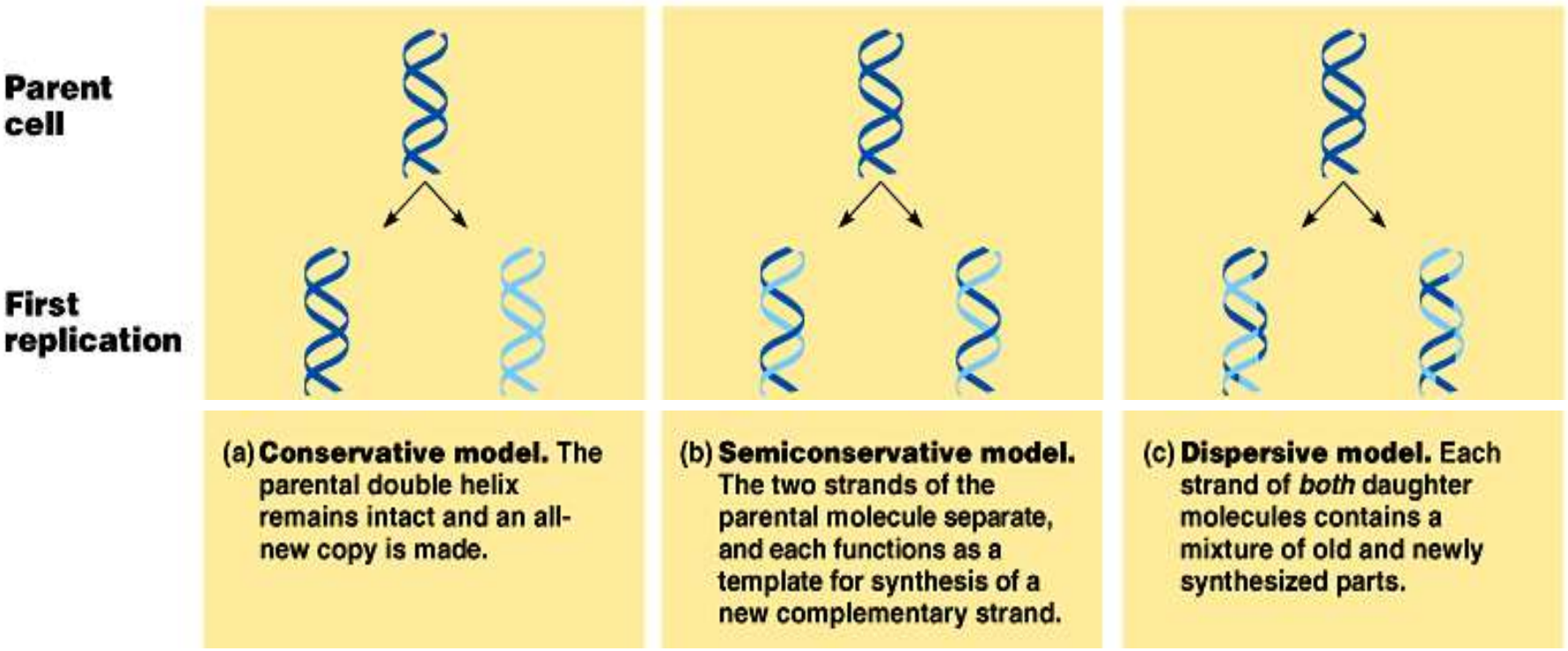
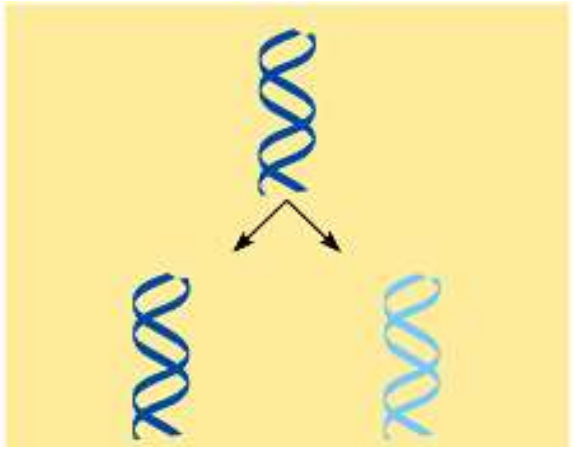
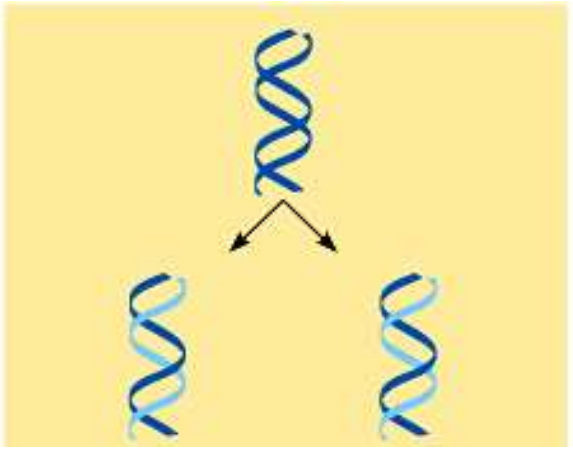
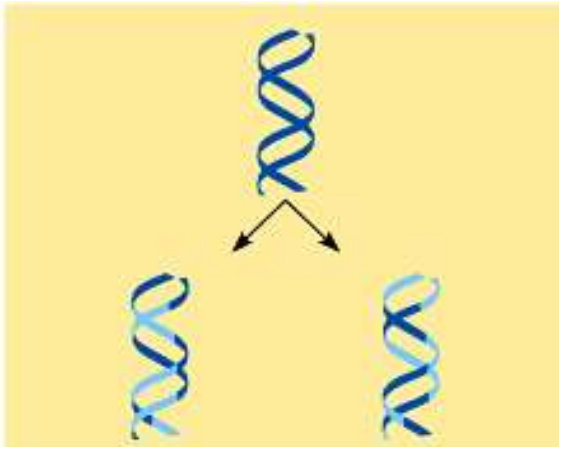


Fig. 16.8

Models of DNA replication







Conservative model	Semiconservative model	Dispersive model
Daughter duplex made of 2 newly synthesized strands. Parent duplex conserved.	Daughter duplexes are made up of one parental strand and one newly synthesized strand	Daughter duplexes are made up of segments of parental DNA and newly synthesized DNA

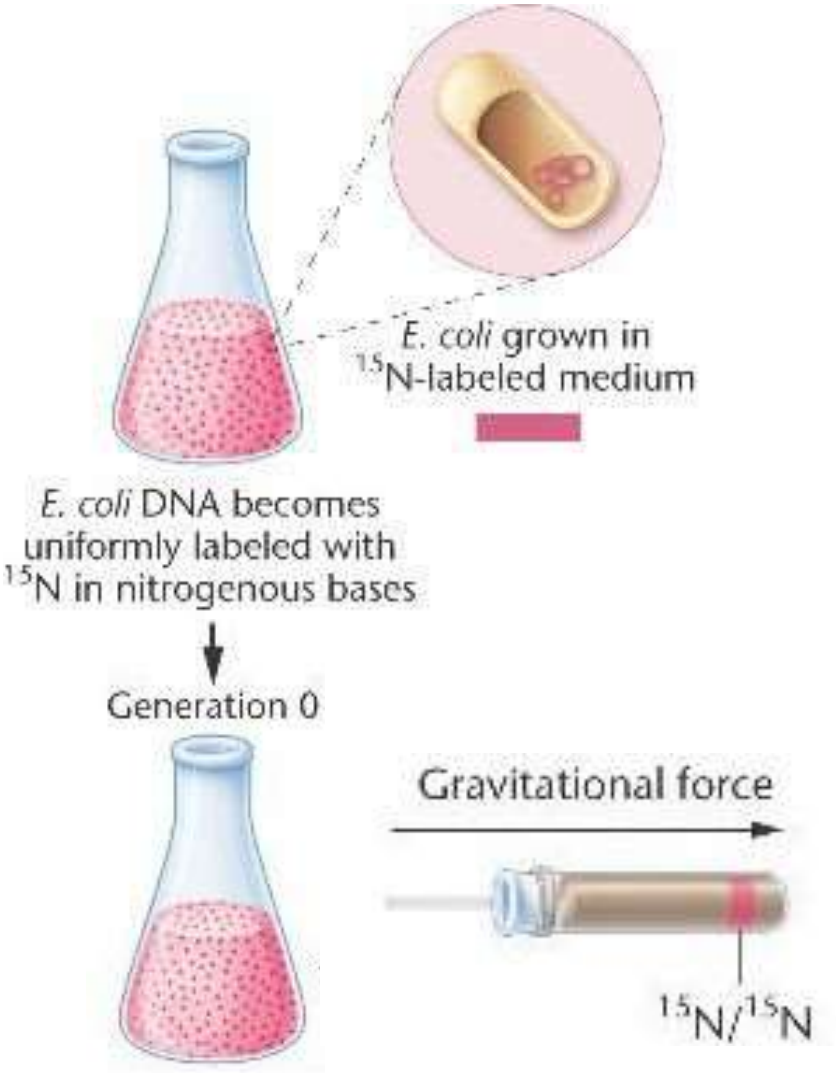
Matthew Meselson & Franklin Stahl (1958)

- Performed an experiment to determine which model of DNA replication was true

See first half of video up to CsCl centrifugation: http://highered.mcgraw-hill.com/olc/dl/120076/bio22.swf

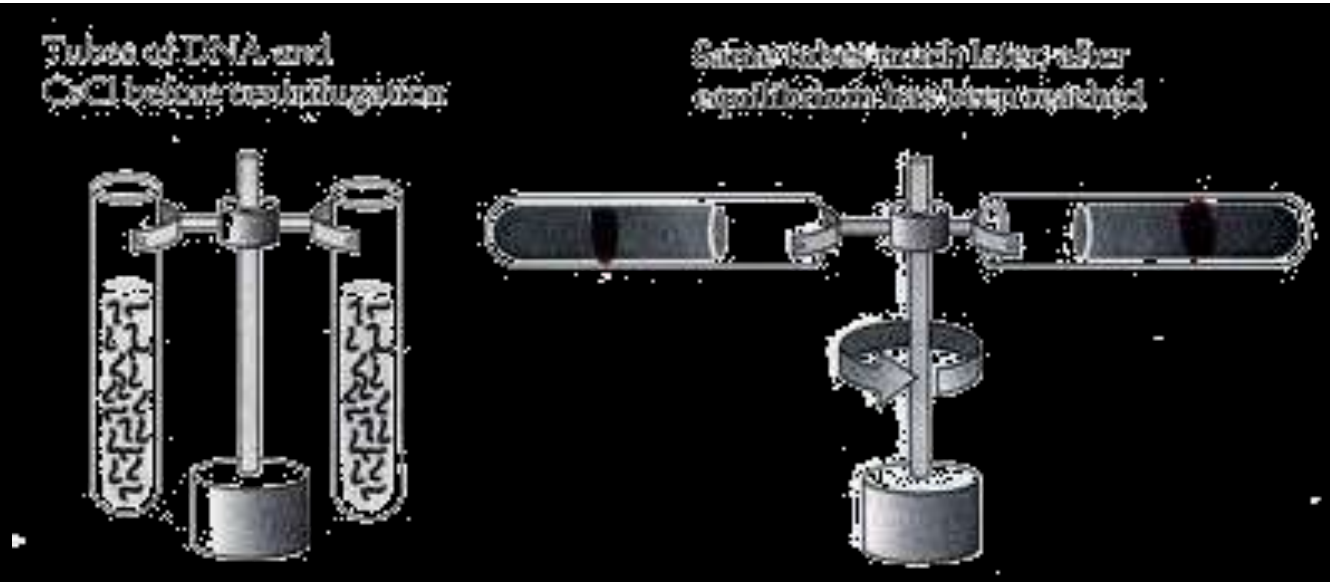
http://www.pnas.org/content/101/52/17889/F1.medium.gif

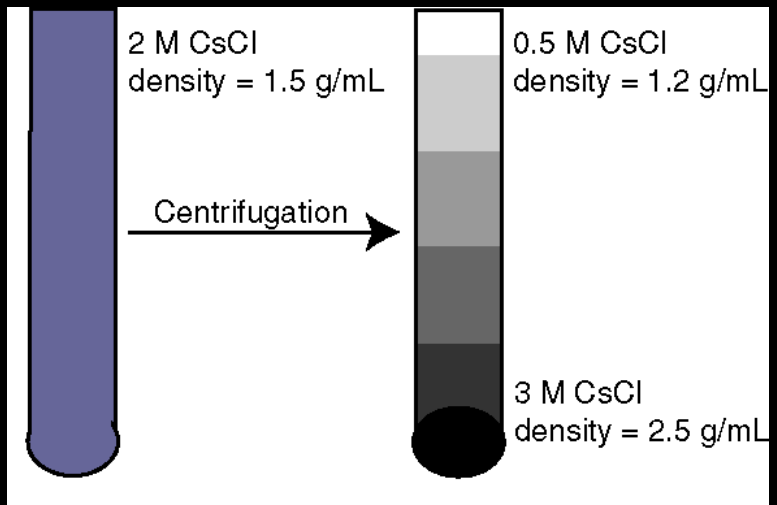
Meselson & Stahl Experiment Step 1



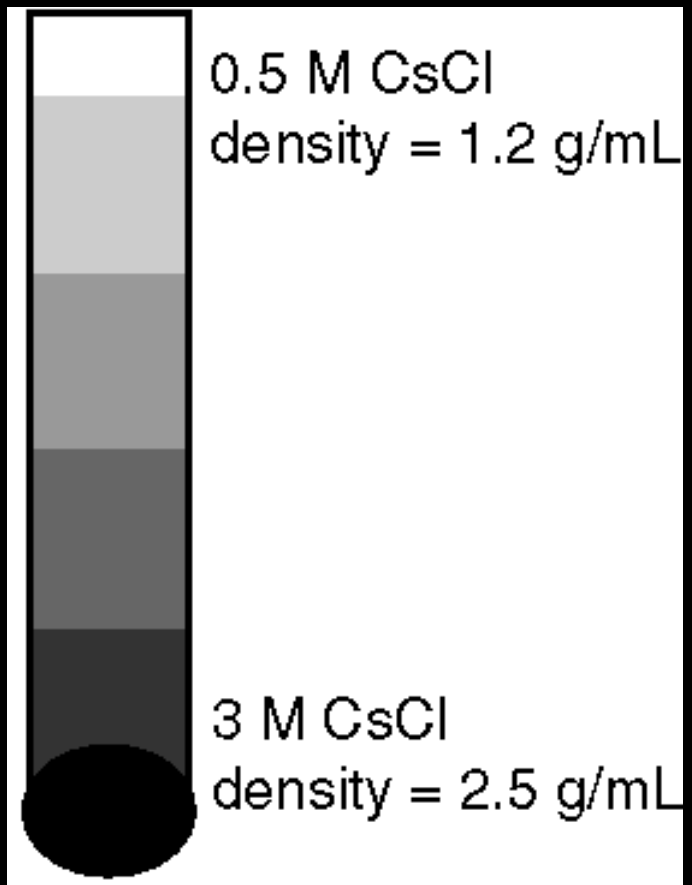
- DNA contains nitrogen atoms (bases)
- First grew E. Coli cells in heavy nitrogen (15N)
- When cells are centrifuged the DNA will show up heavy

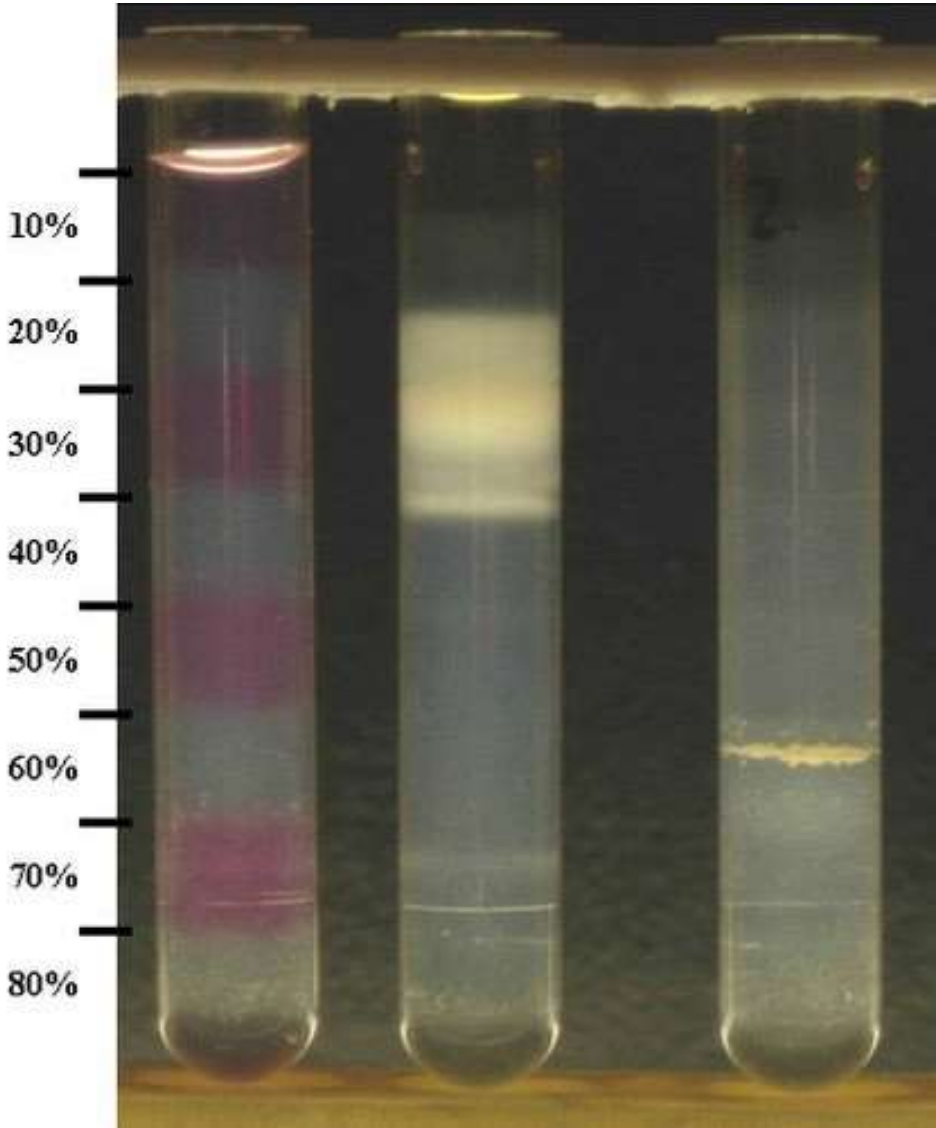
Density Gradient Centrifugation





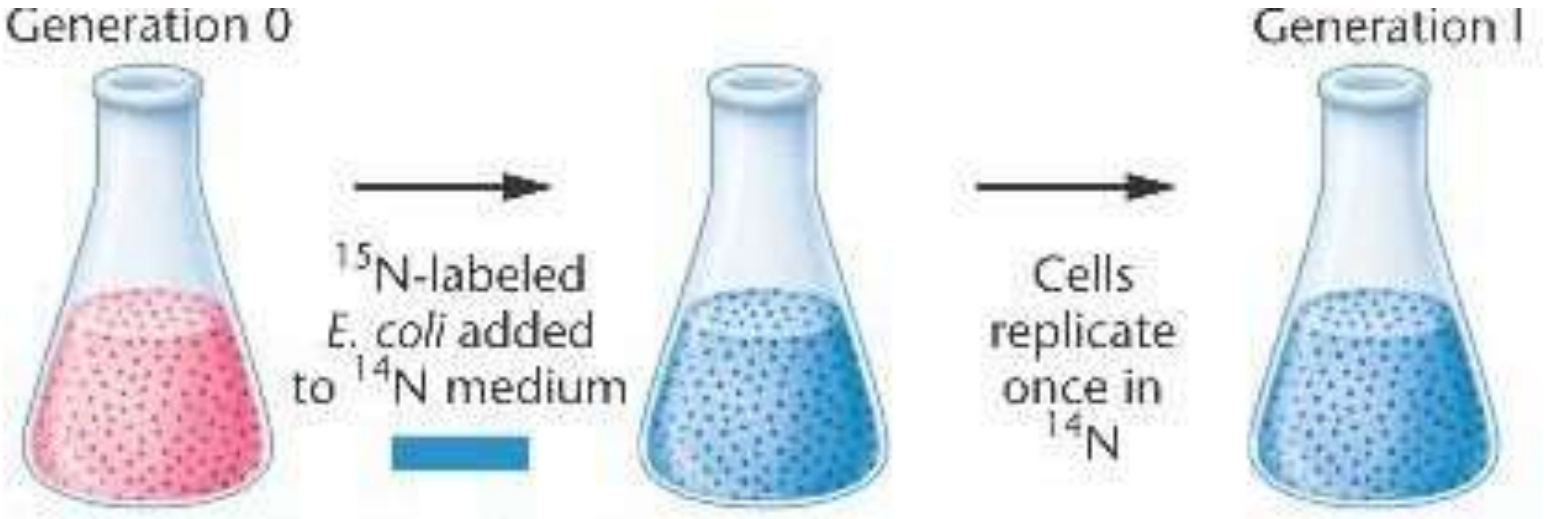
Density Gradient Centrifugation





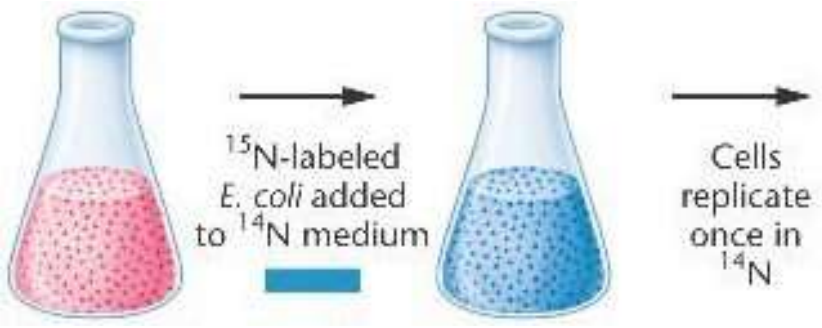
Meselson & Stahl Experiment Step 2

- Then transferred E. Coli cells into a medium with light nitrogen (14N)
- Cells were allowed one round of replication
- Then they were centrifuged



Meselson & Stahl Experiment Step 2

- Predict what the centrifuge tube would show with each model



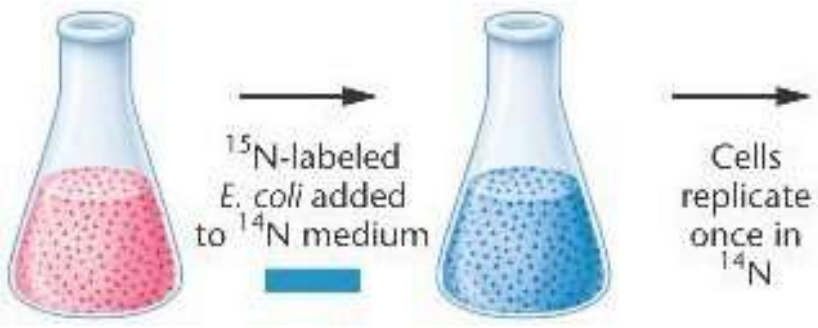
Conservative

Semi-conservative

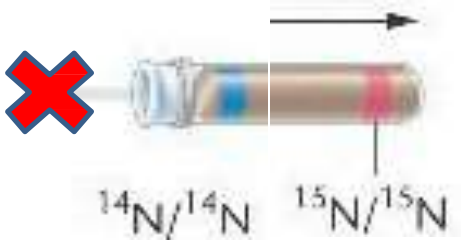
Dispersive

Meselson & Stahl Experiment Step 2

- Which one did he see?



Conservative

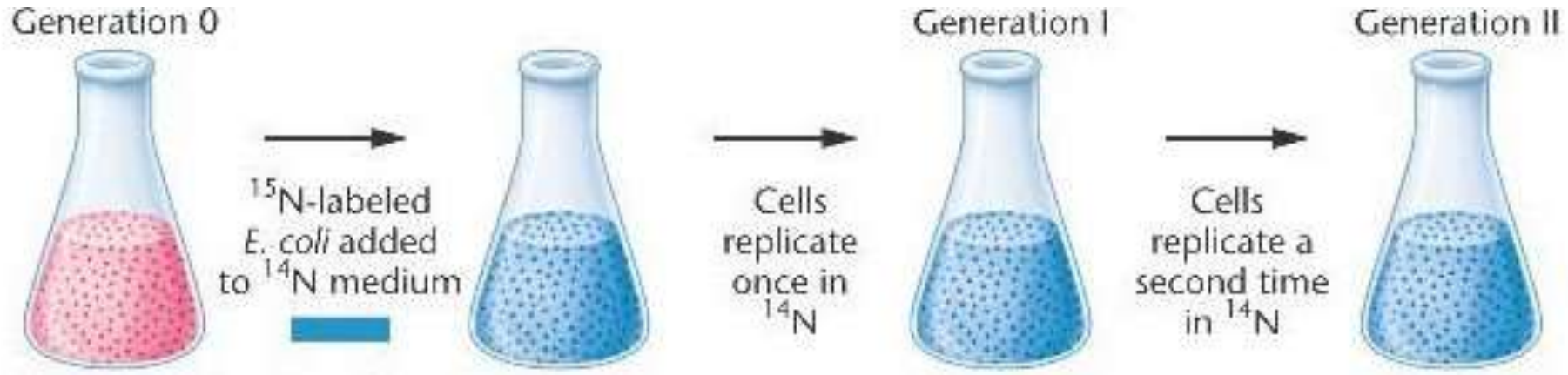


Semi-conservative

Dispersive

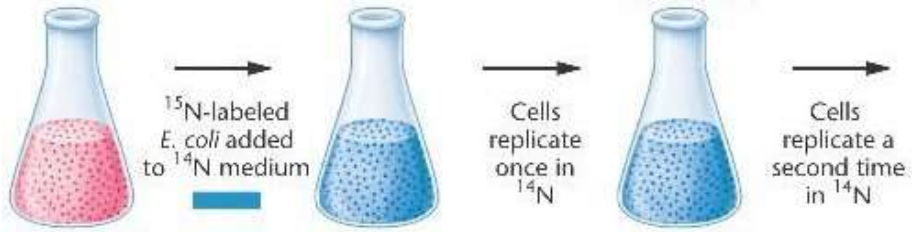
Meselson & Stahl Experiment Step 3

- Cells were then allowed to grow for a second round of replication in the light medium (14N)
- And they were once again centrifuged



Meselson & Stahl Experiment Step 3

- Predict what the centrifuge tube would show with each model



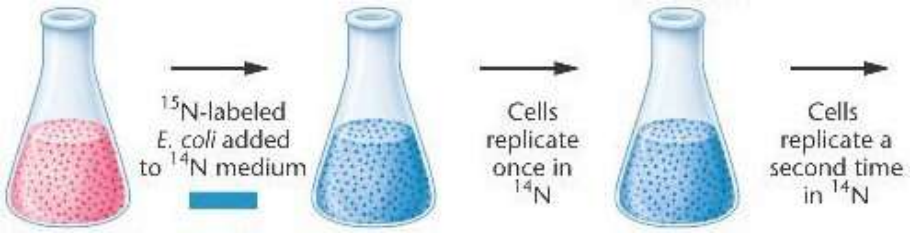
Conservative

Semi-conservative

Dispersive

Meselson & Stahl Experiment Step 3

- Which one did he see?

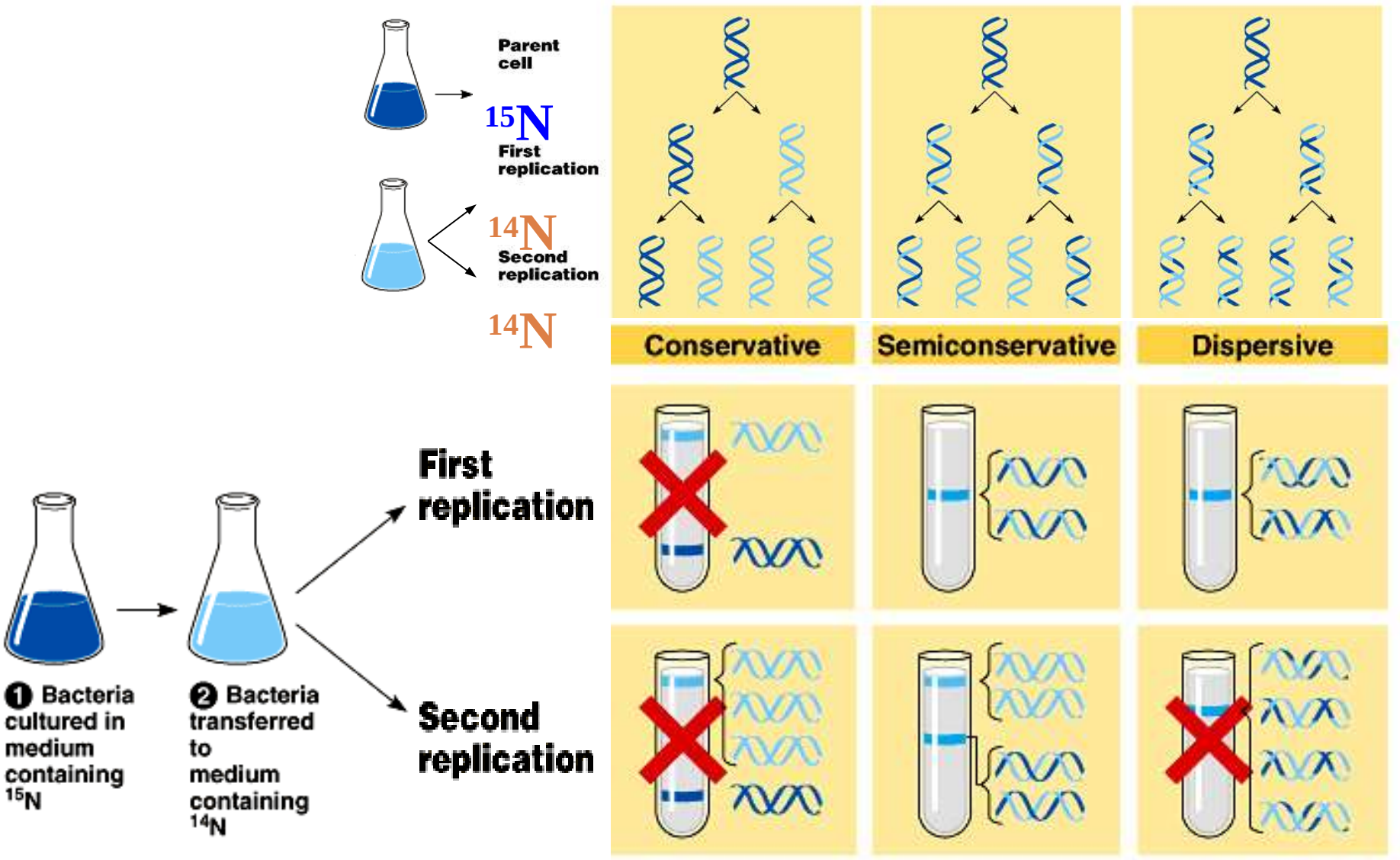


Conservative

Semi-conservative

Dispersive

Meselson & Stahl Experiment Summary

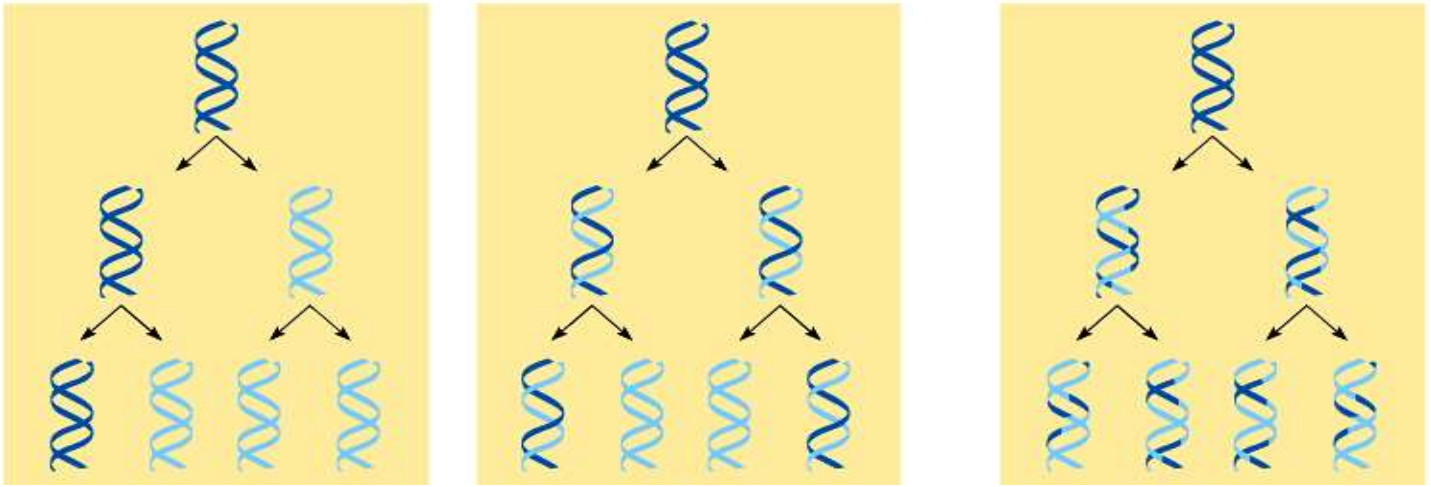


Meselson & Stahl Experiment Summary

Start with ^{15}N
Parent cell

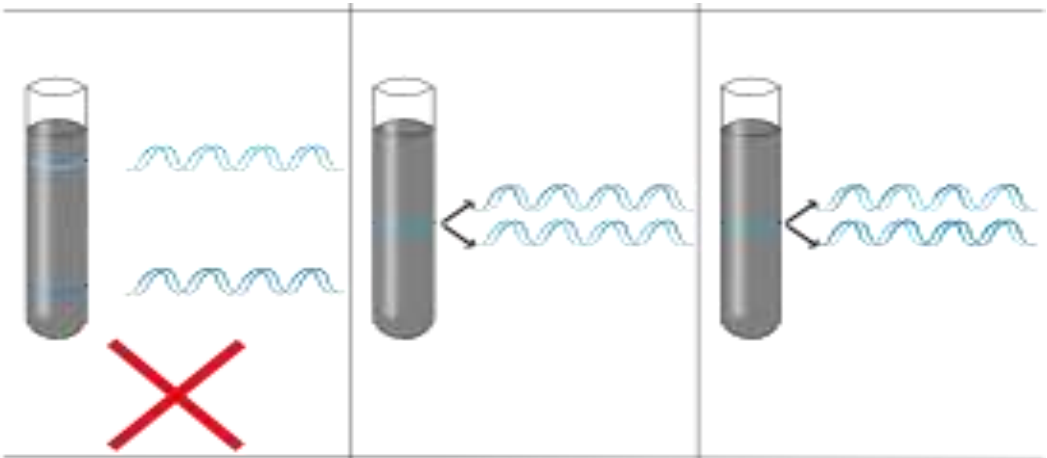
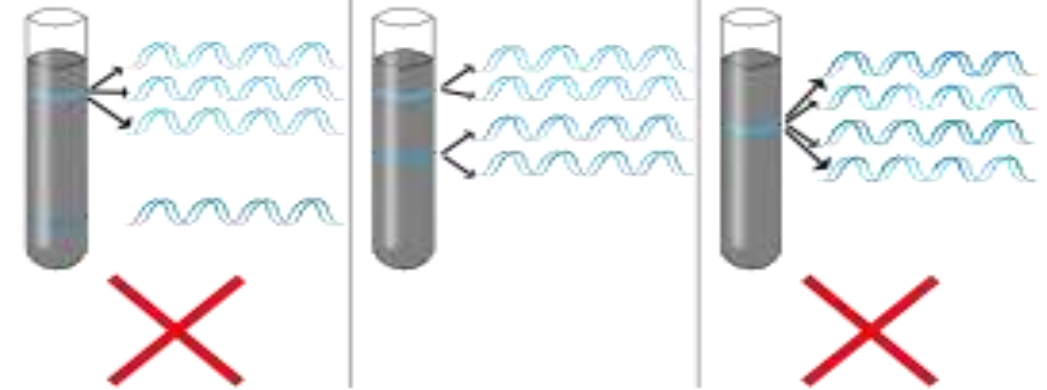
Add ^{14}N Add
First replication

^{14}N
Second replication

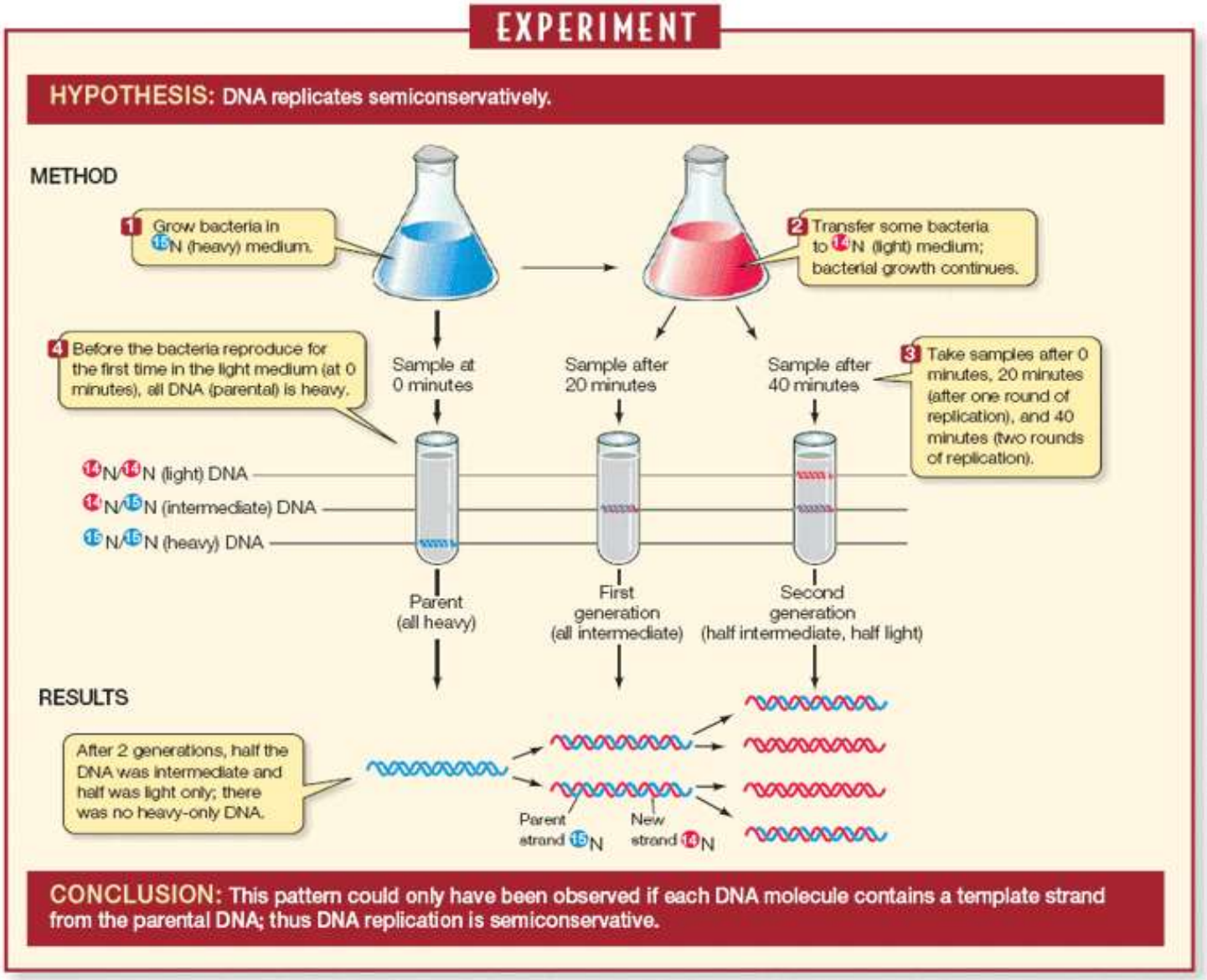


Round of replication	Conservative model	Semiconservative model	Dispersive model
First replication	Two bands Heavy: $^{15}N^{15}N$ Light: $^{14}N^{14}N$	One band medium: $^{15}N^{14}N$	One band medium: $^{15}N^{14}N$
Second replication	Two bands Heavy: $^{15}N^{15}N$ Light: $^{14}N^{14}N$	Two bands Medium: $^{15}N^{14}N$ Light: $^{14}N^{14}N$	One band at a weight that is in between the medium and light band weights

Meselson & Stahl Experiment Summary

Model	Conservative	Semi-conservative	Dispersive
Generation I			
Generation II			

Meselson & Stahl Experiment



Basic concept of DNA replication

(video)

http://www.hhmi.org/biointeractive/dna/DNAi_replication_schematic.html

Basic concept of DNA replication

- During DNA replication, base pairing enables existing DNA strands to serve as templates for new complimentary strands

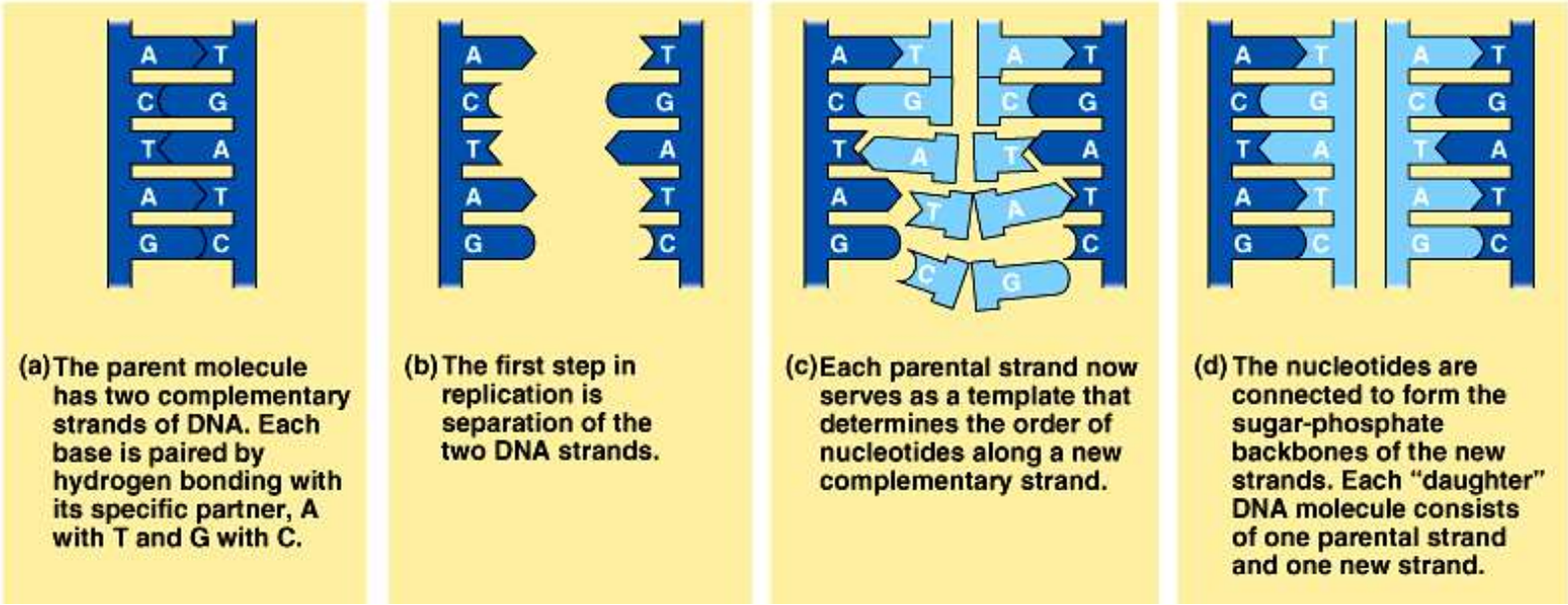


Fig. 16.7

2. DNA Replication Mechanism

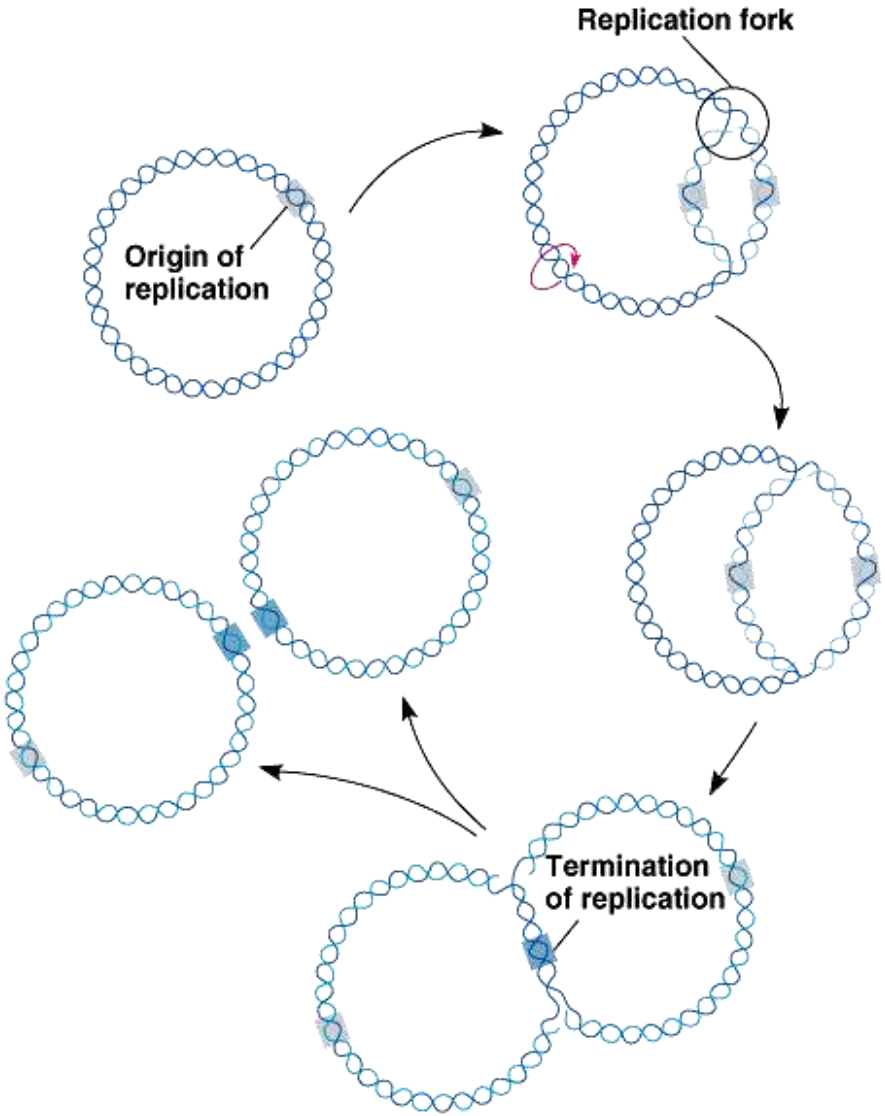
- Initiation
- Elongation
- Termination

DNA replication: Initiation

- **Origins of replication (ori)**: Special sites on DNA where replication begins

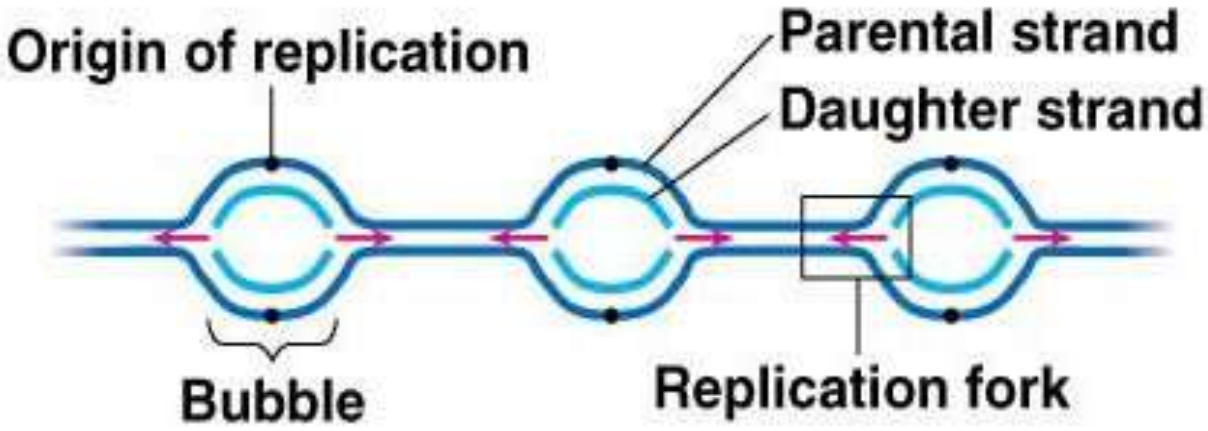
Replication Initiation: Prokaryote

- Replication begins at one fixed origin (only 1 ori)
- Replication proceeds bidirectionally until the DNA is replicated



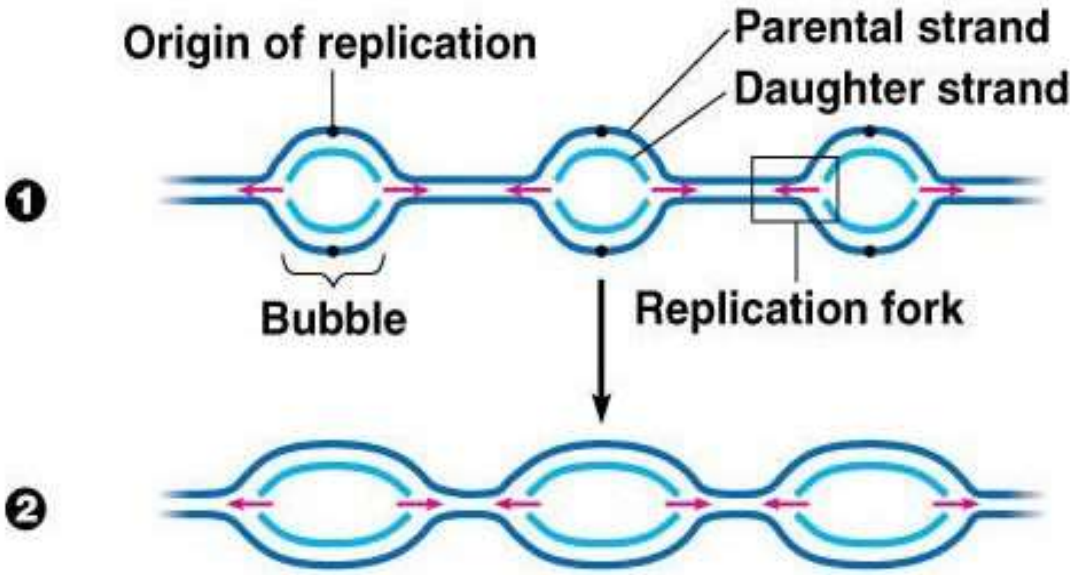
Replication Initation: Eukaryote

- More than one origin of replication (thousands of origin sites per chromosome)



Replication forks and bubbles

- At the origin sites, the DNA strands separate forming a replication "bubble" with replication forks at each end.



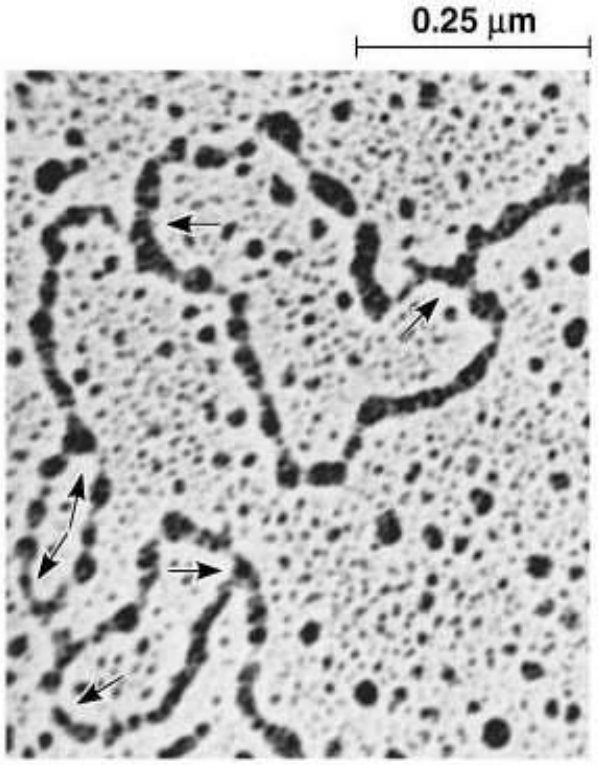


Fig. 16.10

Replication forks and bubbles

- The replication bubbles elongate as the DNA is replicated and eventually fuses.

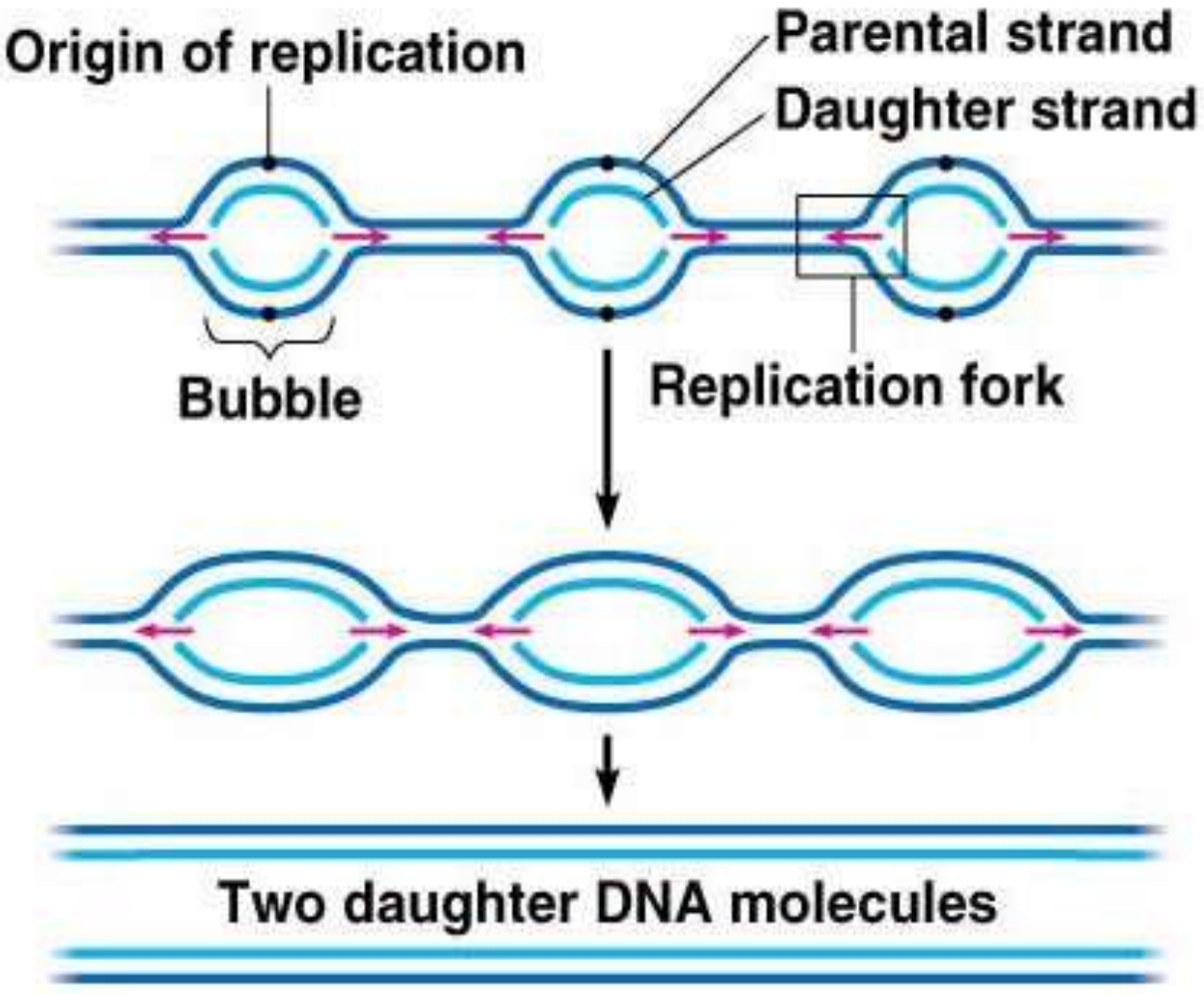


Fig. 16.10

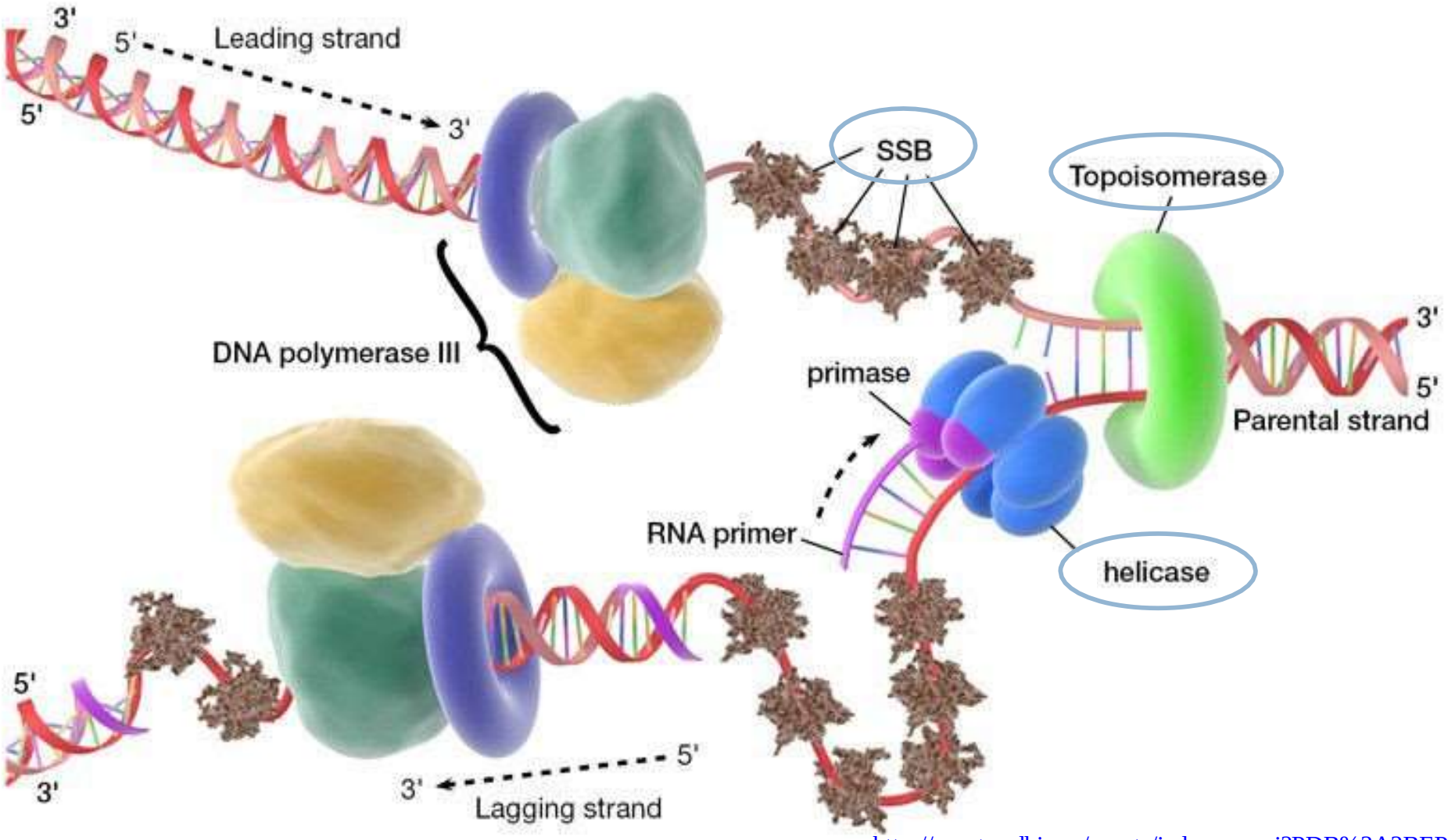
Proteins in Replication Initiation

- Sticky Tack Demo to show action of helicase, SSBP and topoisomerase
- Yarn
- Sticky tack or tape
- scissors

- Watch first half of video (before the fork):

http://highered.mcgraw-hill.com/olc/dl/120076/bio23.swf

Proteins in Replication Initiation

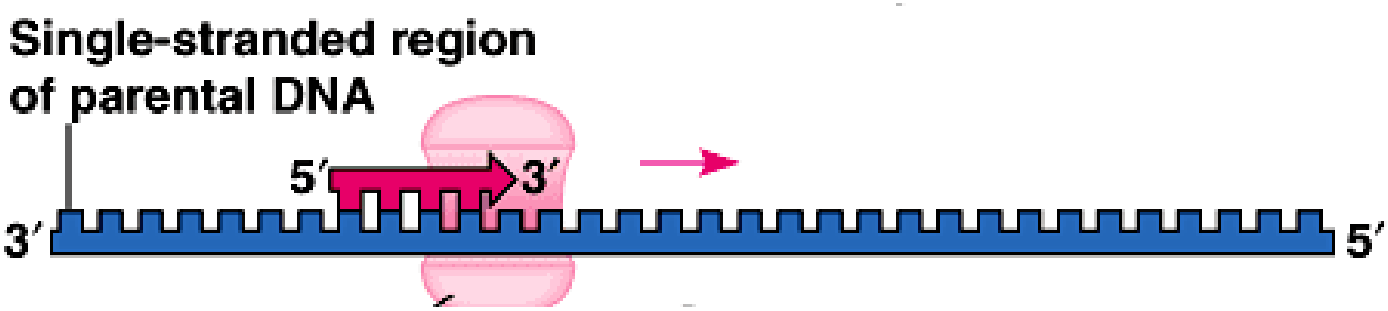


http://eprots.pdbj.org/eprots/index_en.cgi?PDB%3A3BEP

Proteins in Replication Initiation

- **Helicase**: enzyme that disrupts H bonds between two strands of DNA to separate the template DNA strands at the replication fork.
- **Single-strand binding proteins (SSBPs)**: proteins that bind to unwound single-stranded regions of DNA to keep the template strands apart during replication
- **Topoisomerases**: enzymes that can break bonds in DNA and then reforms the bonds
  - Purpose is to release the twists in DNA that are generated during DNA replication
  - Example of a topoisomerase: **DNA gyrase**

Priming DNA for replication



- **Blue line:** DNA to be copied
- **Pink line:** RNA nucleotides added = Primer
- **Light pink blob:** Enzyme that adds RNA = RNA polymerase

Fig. 16.13

Priming DNA for replication

- **Primer**: a short segment of RNA needed to initiate DNA replication
- Note: all nucleic acids are formed in the 5′ to 3′ direction, even RNA (thus primers)
- **Primase**: an RNA polymerase (RNAP) which synthesizes the primer by adding ribonucleotides that are complementary to the DNA template
- Polymerase: enzyme that makes polymers

Why is priming required?

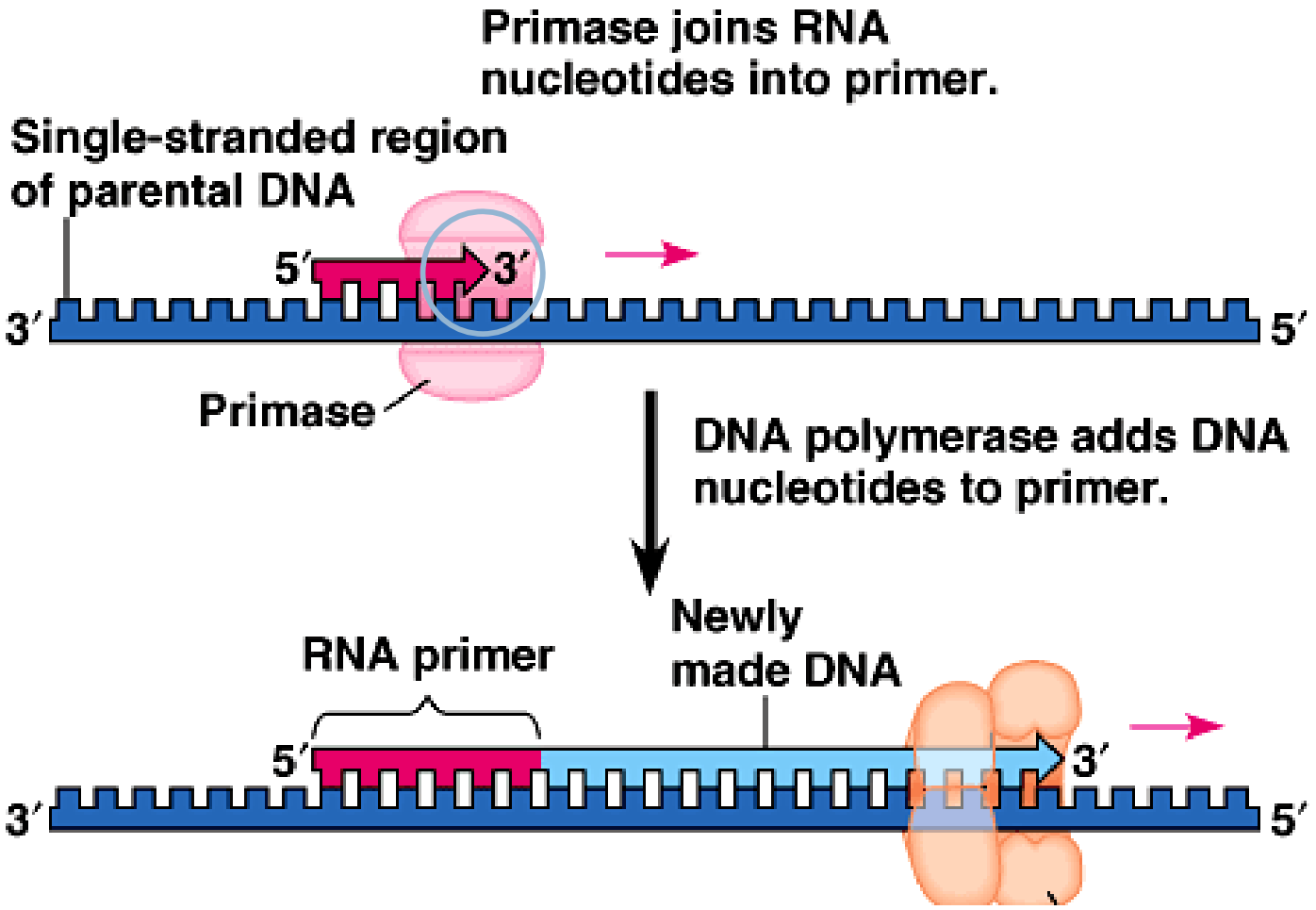


Fig. 16.13

Why is priming required?

- Due to the different abilities of RNA polymerase (RNAP) versus DNA polymerase (DNAP)
- RNAP:
- can start a new chain without an existing end
- All it needs is a template
- E.g. primase
- DNAP:
- can only add nucleotides to the end of an existing chain
- can never start a new chain because it needs the 3′ OH

DNA Polymerase (DNAP)

- Enzyme which synthesizes nucleotide chains
- Prokaryotes:
  - DNA polymerase I, II, III, IV & V
- Eukaryotes:
  - over 15 different types named with Greek letters (e.g. DNAP α)

DNA Replication: Elongation

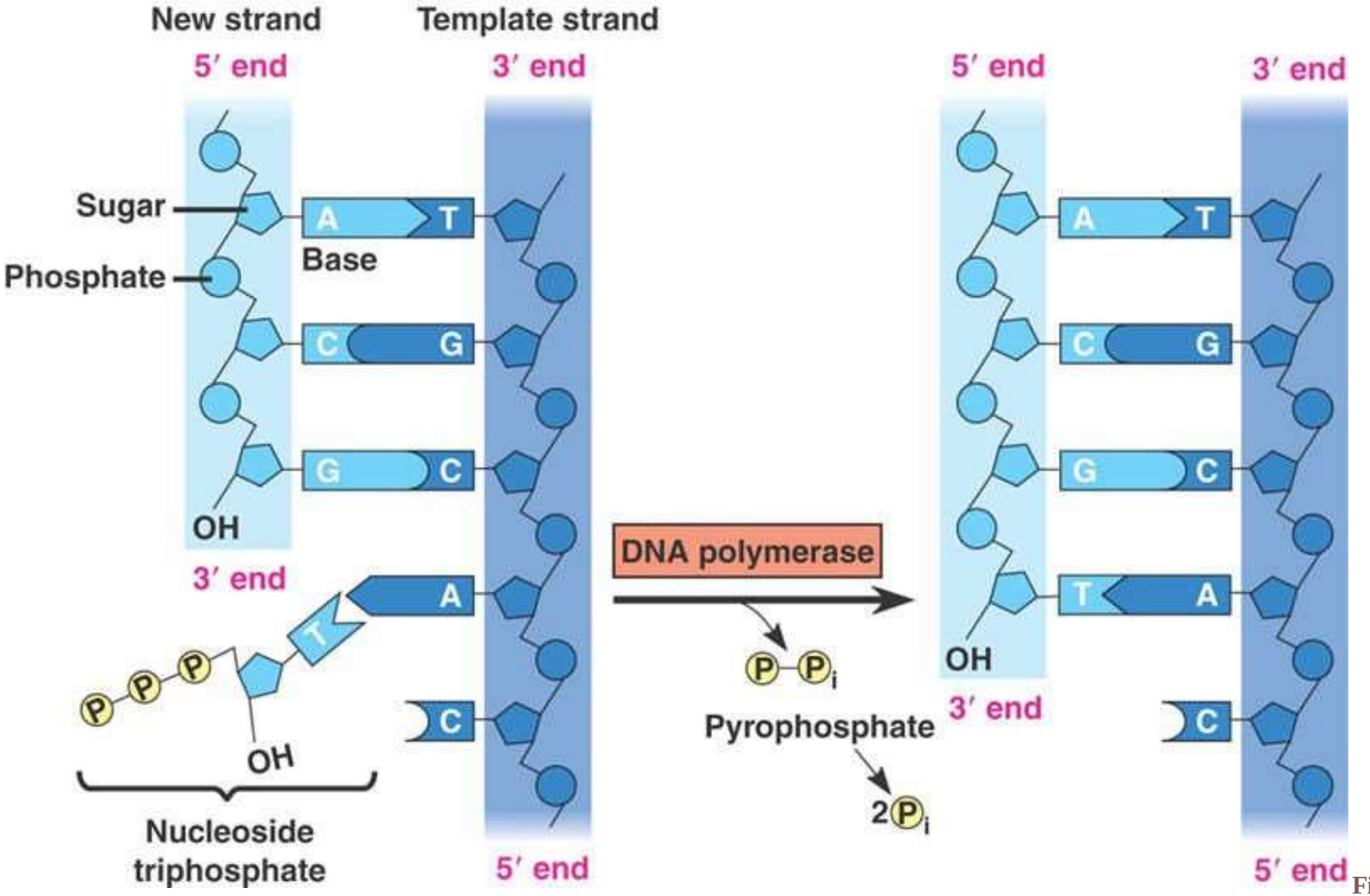
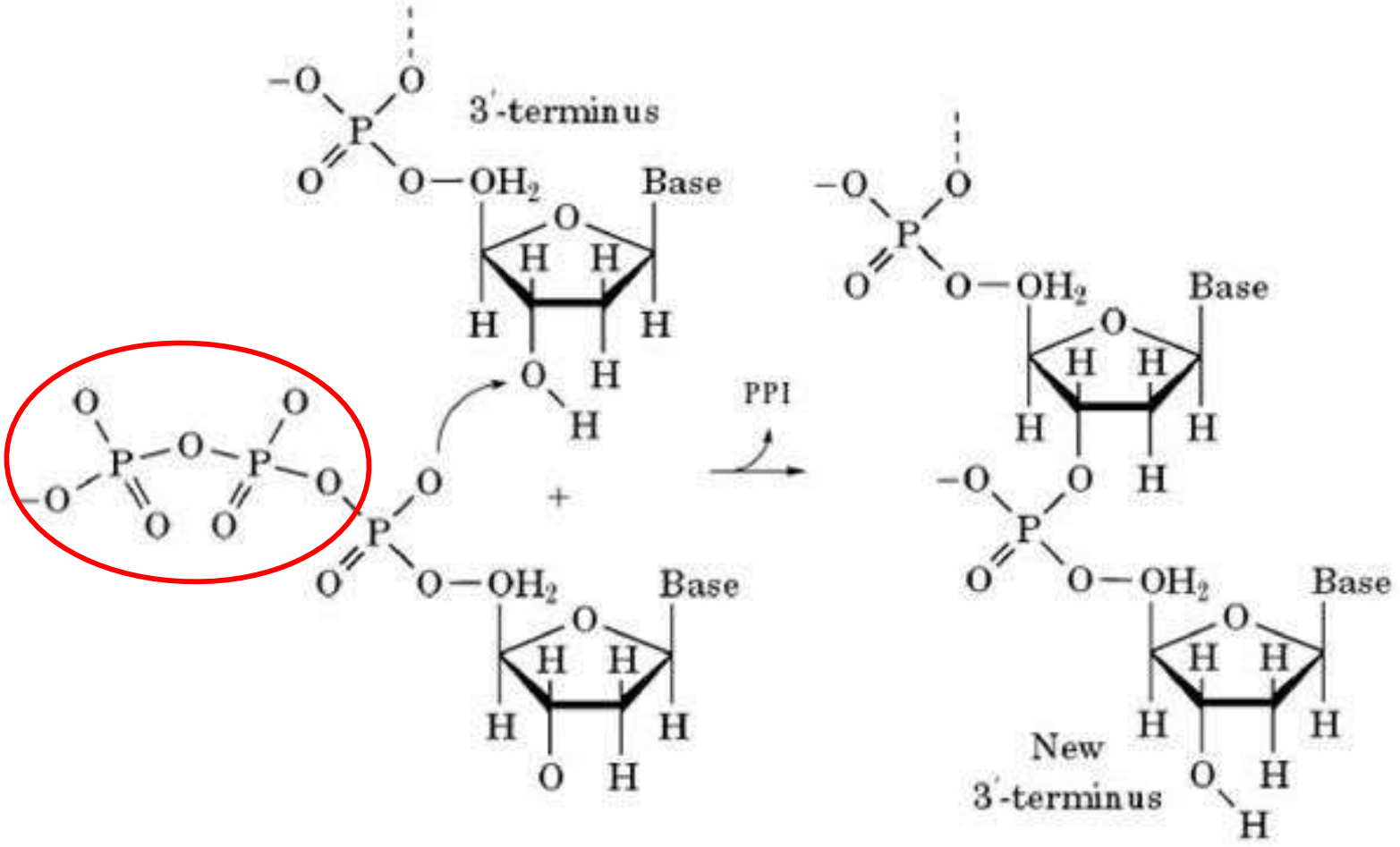


Fig. 16.11

DNA Replication: Elongation

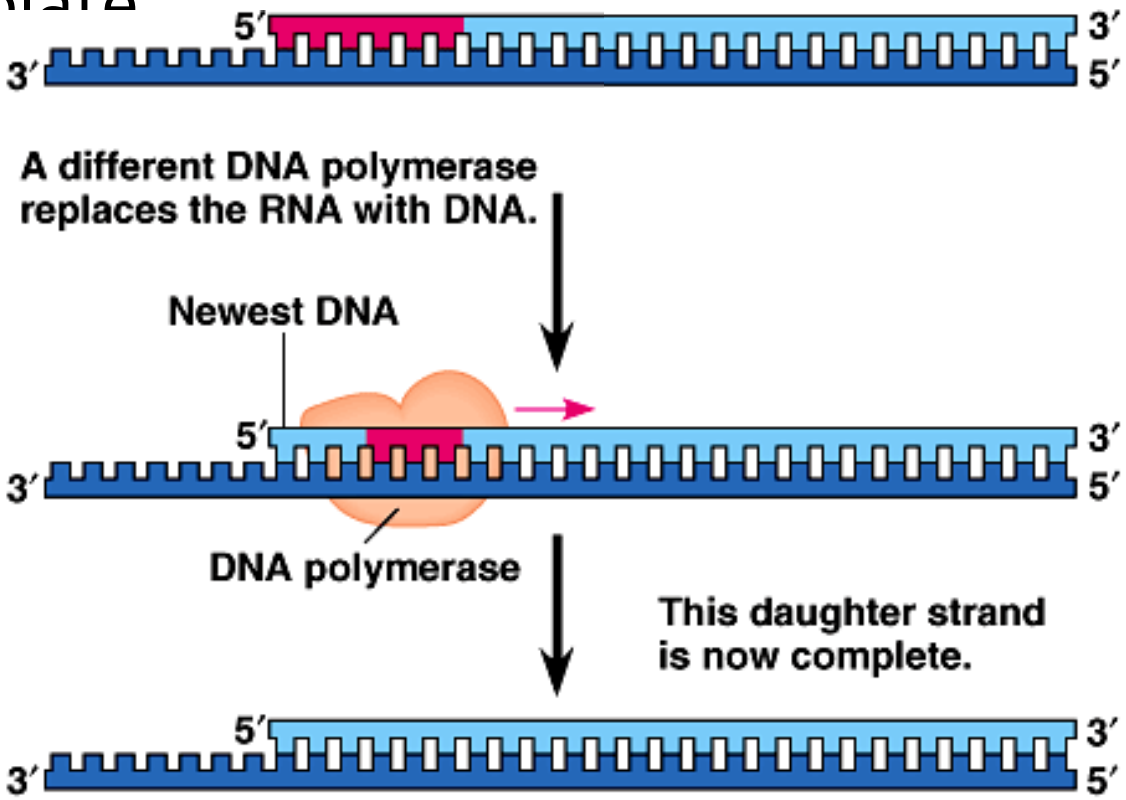
- DNA polymerase III (DNAP III) catalyzes the elongation of DNA molecules by adding nucleotides to the 3′ end of a pre-existing nucleotide
- As each nucleotide is added, the last two phosphate groups are hydrolyzed to form pyrophosphate.
- Pyrophosphate is broken down into two phosphates
- NTP · NMP + 2P

DNA Elongation



DNA Polymerase

- **DNA polymerase I** (DNAP I) replaces the RNA primer with DNA complementary to the template



DNA Elongation

DNAP III: elongates DNA strand

DNAP I: replaces RNA with DNA

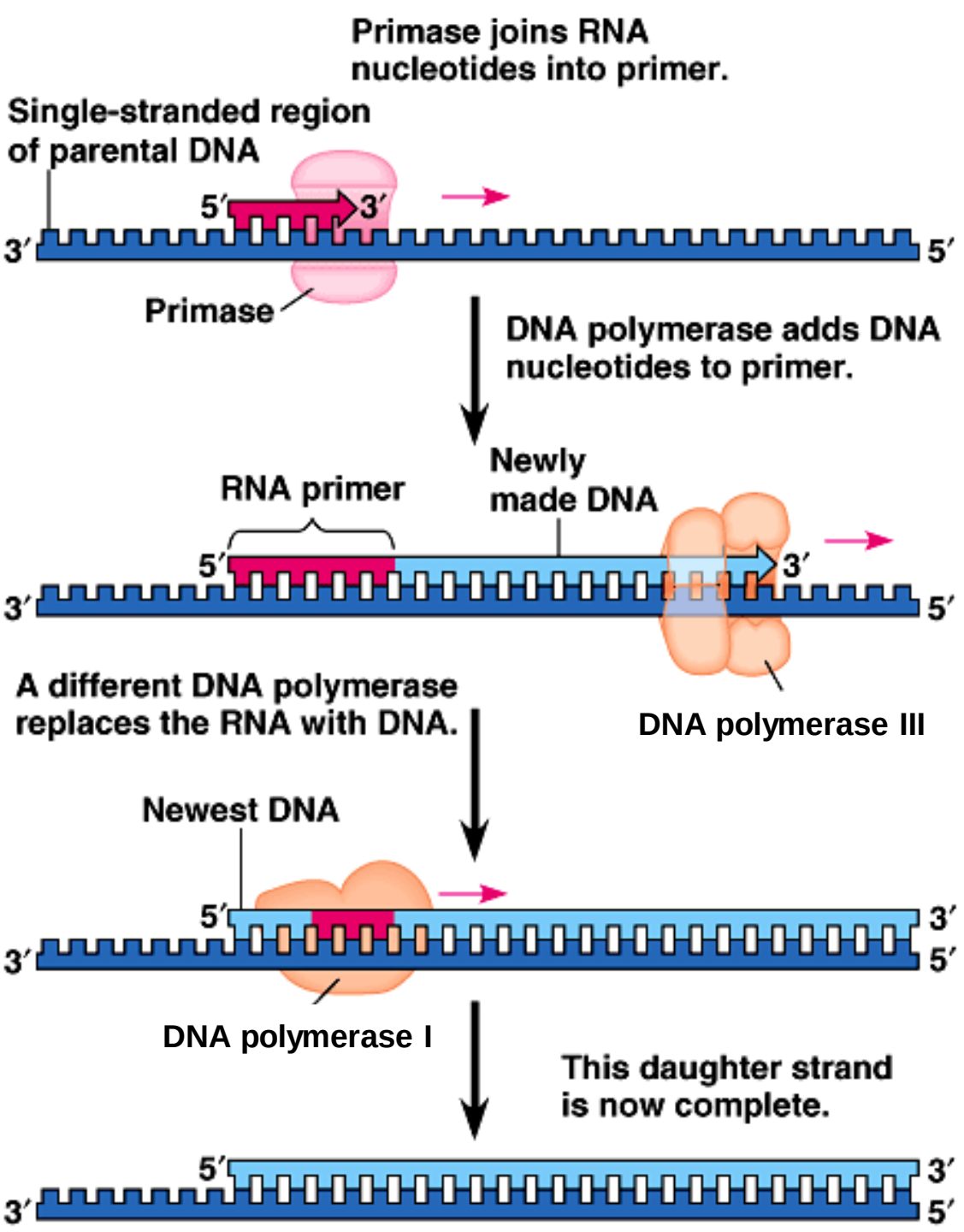
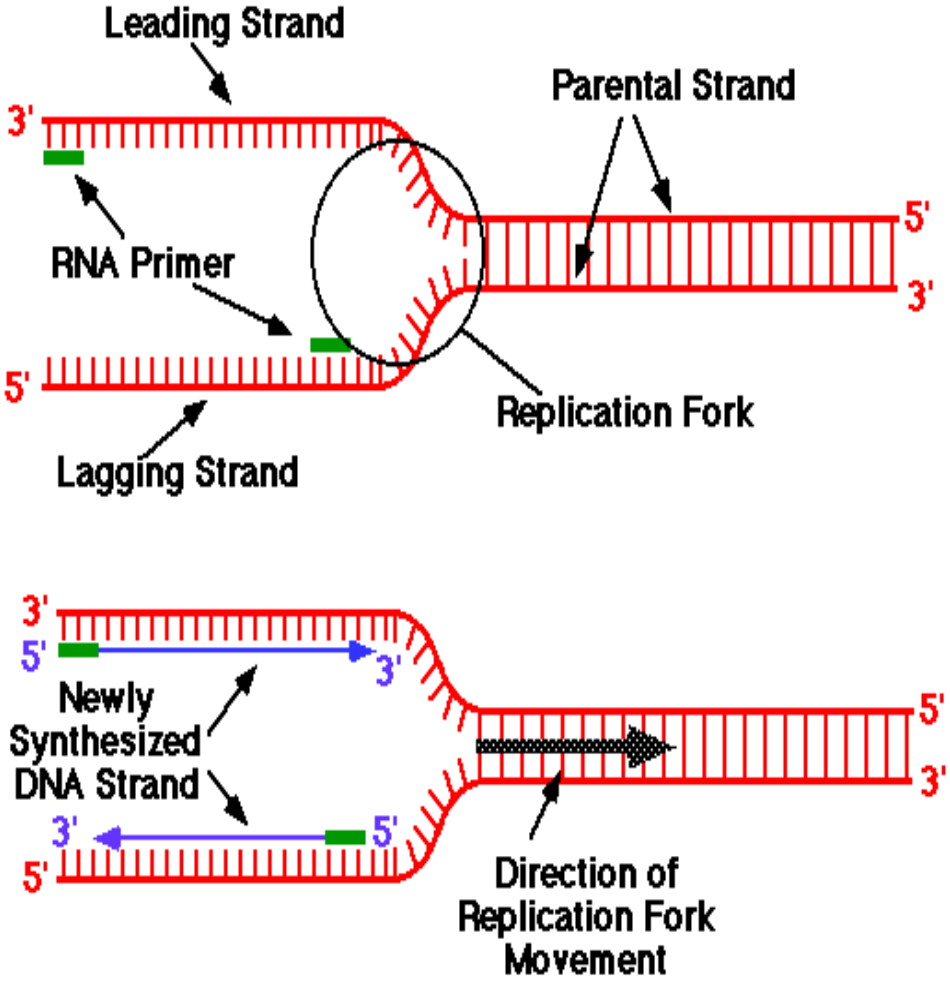


Fig. 16.14

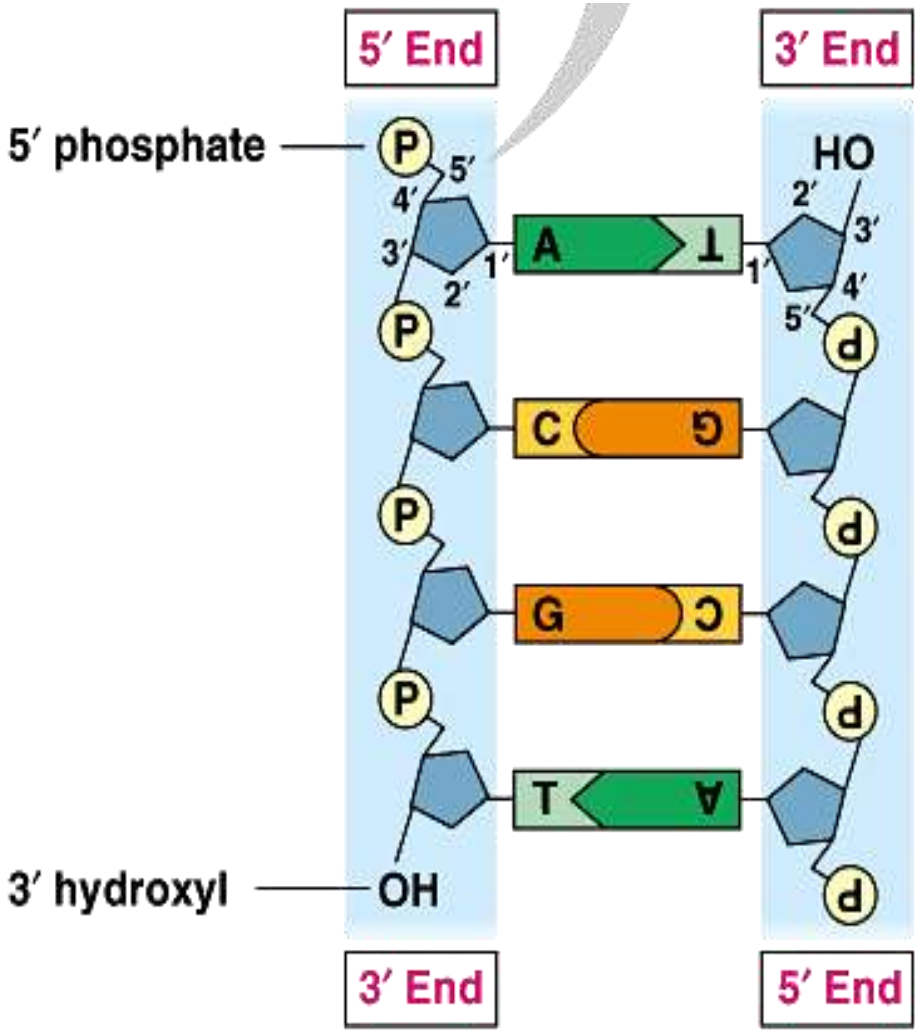
The problem at the fork

- Due to antiparallel nature of DNA
- One parental strand has its 3′ end at the fork while the other parental strand has its 5′ end at the fork.
- But DNA synthesis can only proceed in a 5′ · 3′ direction.



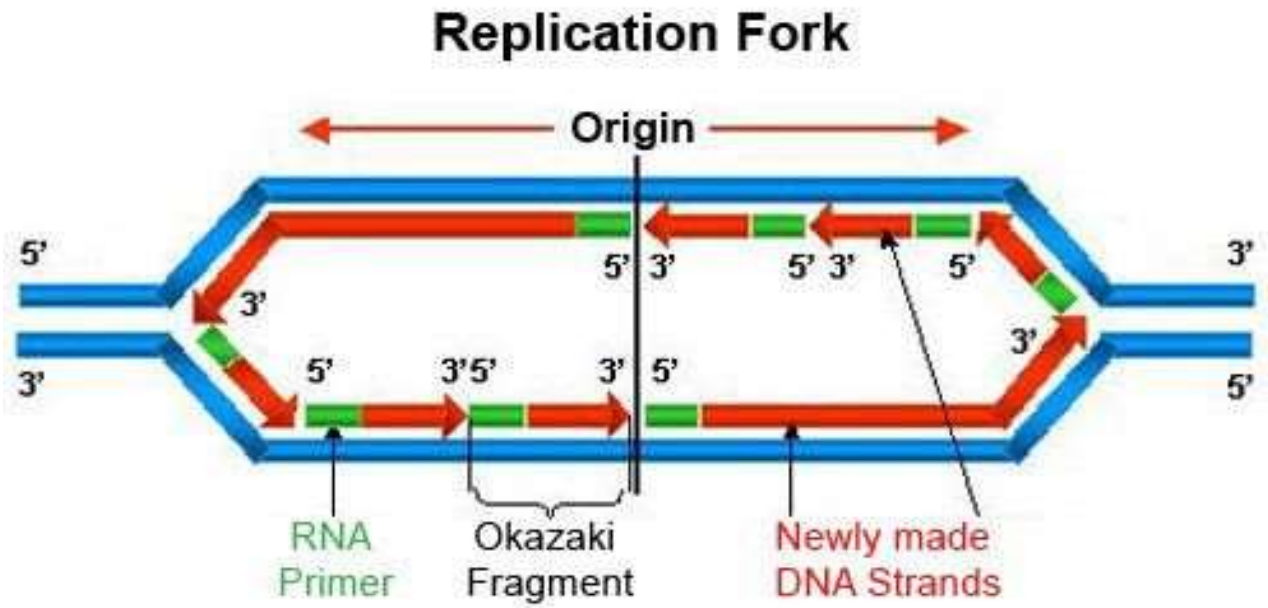
Directionality

- A strand of DNA can only add nucleotides onto its 3′ end
- DNA elongation only proceeds in the 5′ to 3′ direction
- DNAP must move along the template strand's 3′ to 5′ direction.

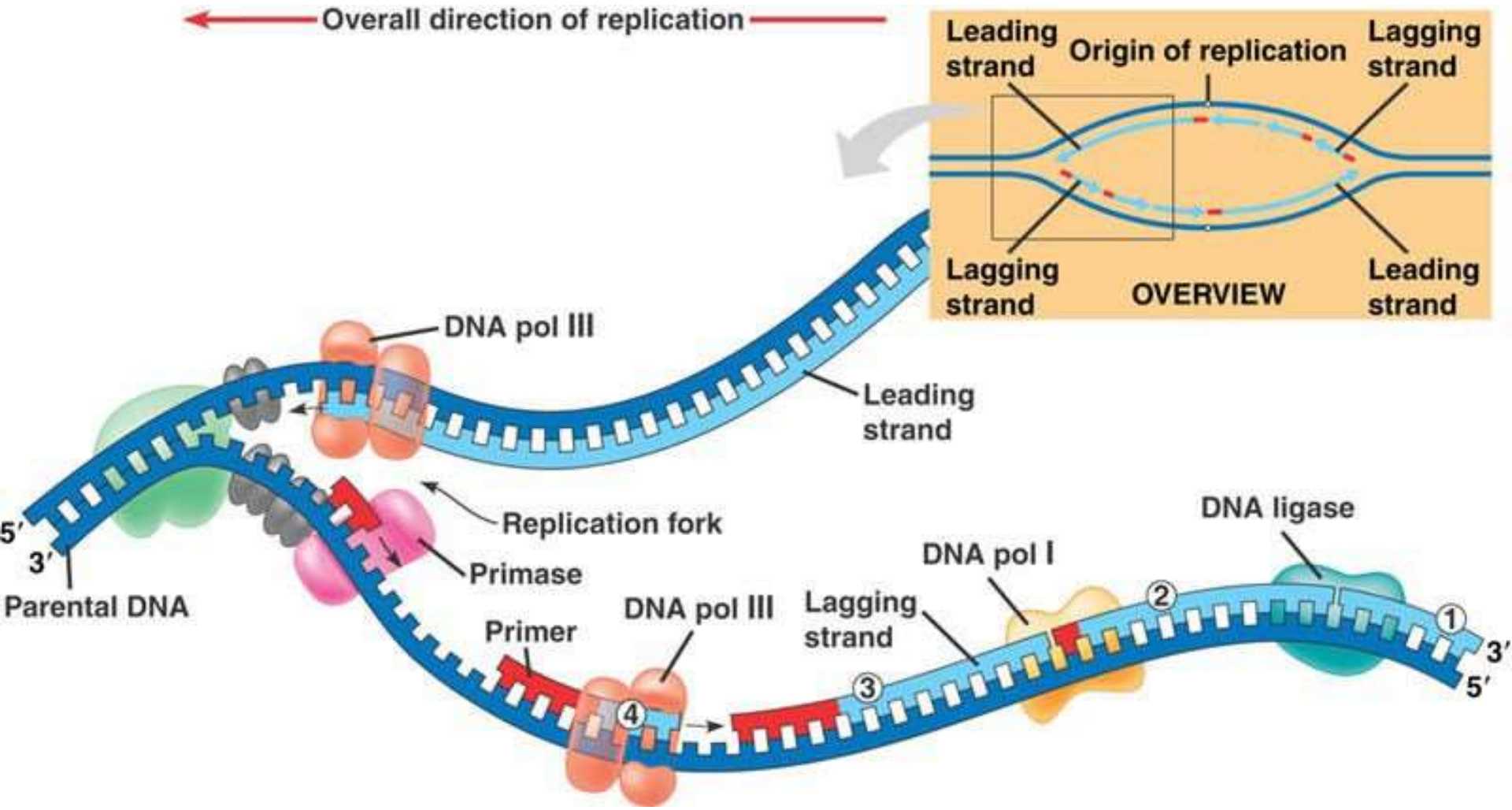


The leading and lagging strands

- **Leading strand**: is synthesized continuously
- **Lagging strand**: is synthesized in short, discontinuous segments of 1000-2000 nucleotides called **Okazaki fragments**



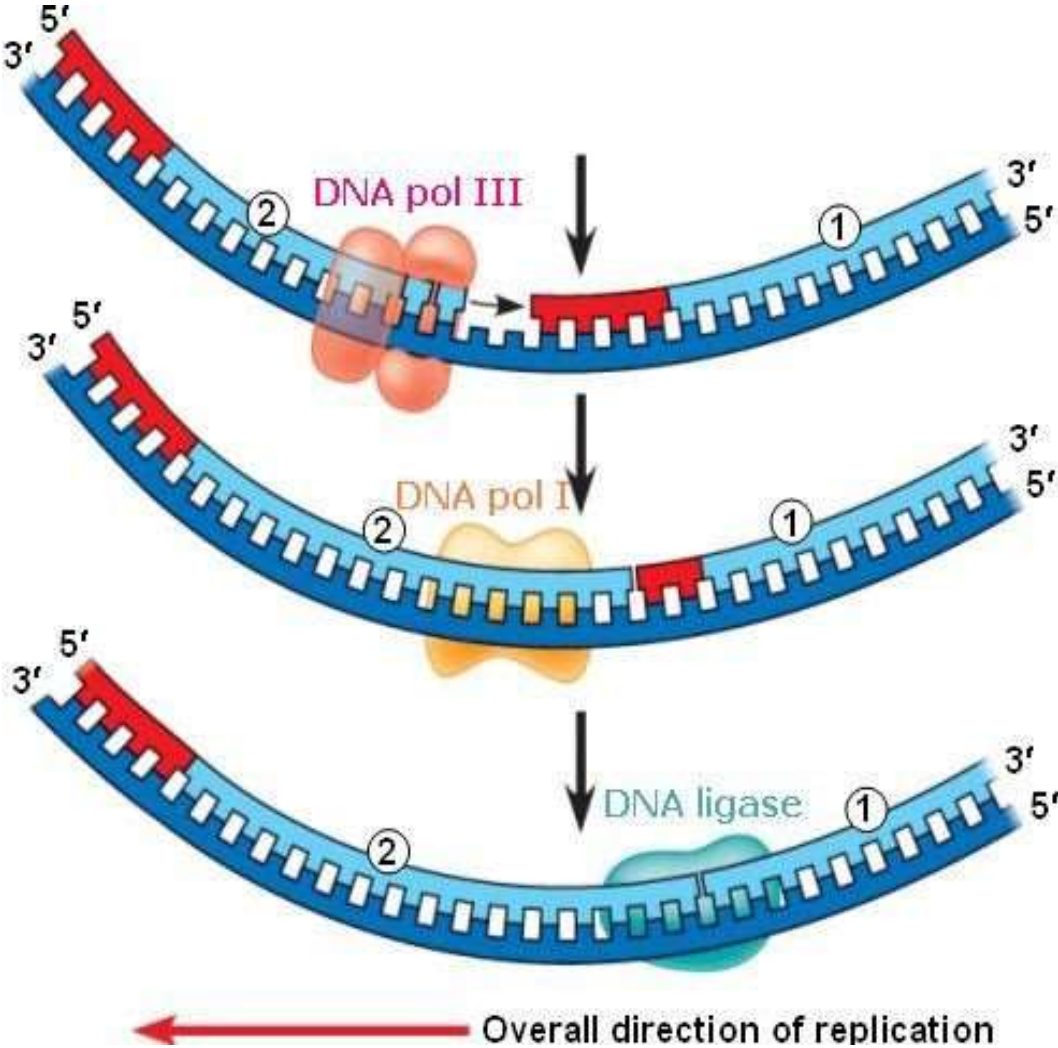
Replication Fork



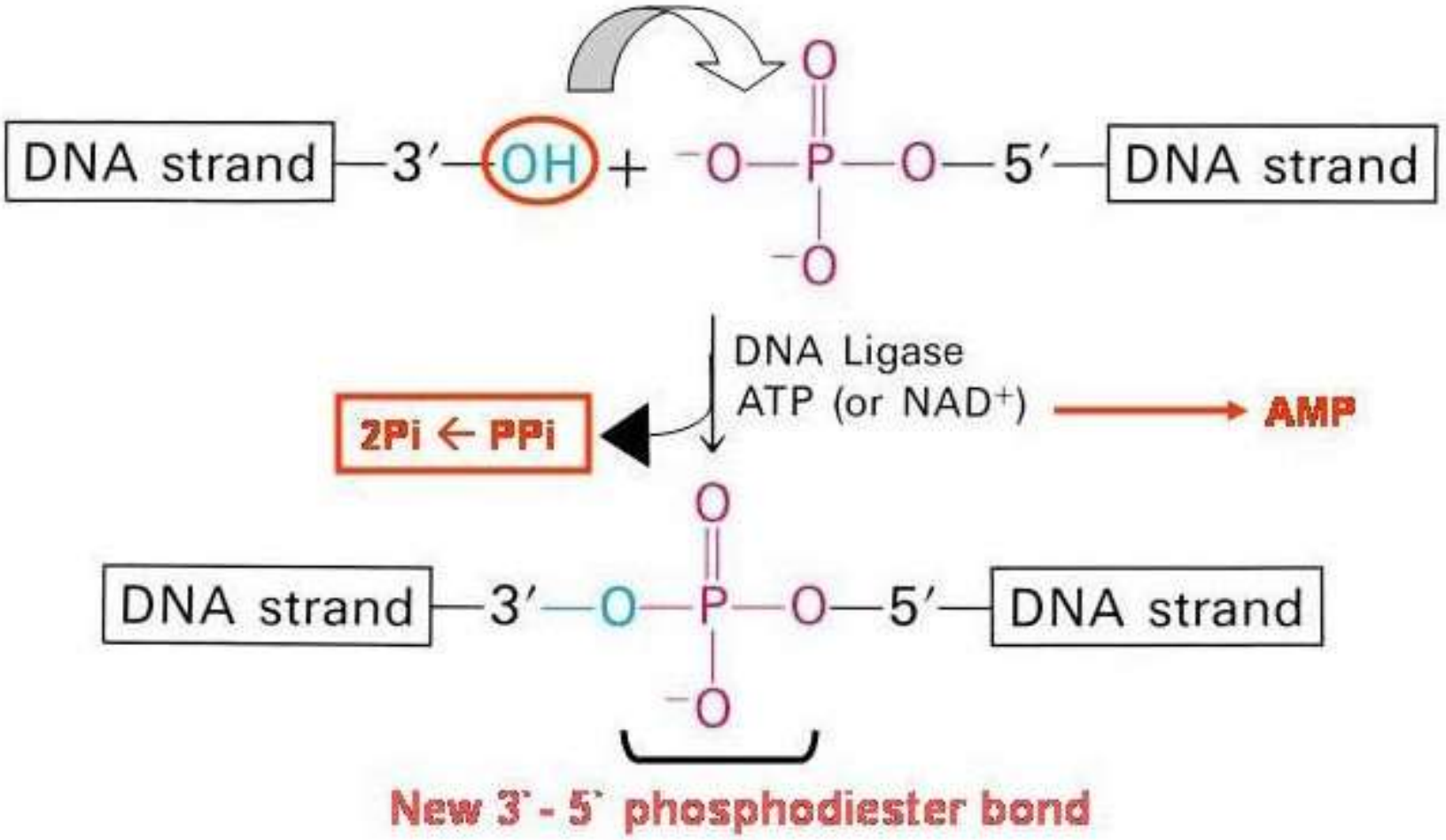
http://kvhs.nbed.nb.ca/gallant/biology/replication_overview.jpg Fig. 16.16

Lagging strand: Okazaki fragments

- **DNAP III** synthesizes the DNA
- **DNAP I** replaces the RNA primer with DNA complementary to the template
- **DNA ligase** joins broken pieces of DNA by catalyzing the formation of phosphodiester bonds



DNA Ligase



http://fhs-bio-wiki.pbworks.com/f/DNA%20Ligase%20reaction.jpg

Animations: Replication at the Fork, Leading & Lagging Strands

- Second half of video: http://highered.mcgraw-hill.com/olc/dl/120076/bio23.swf
- Video: http://highered.mcgraw-hill.com/olc/dl/120076/micro04.swf

DNA Replication Machinery

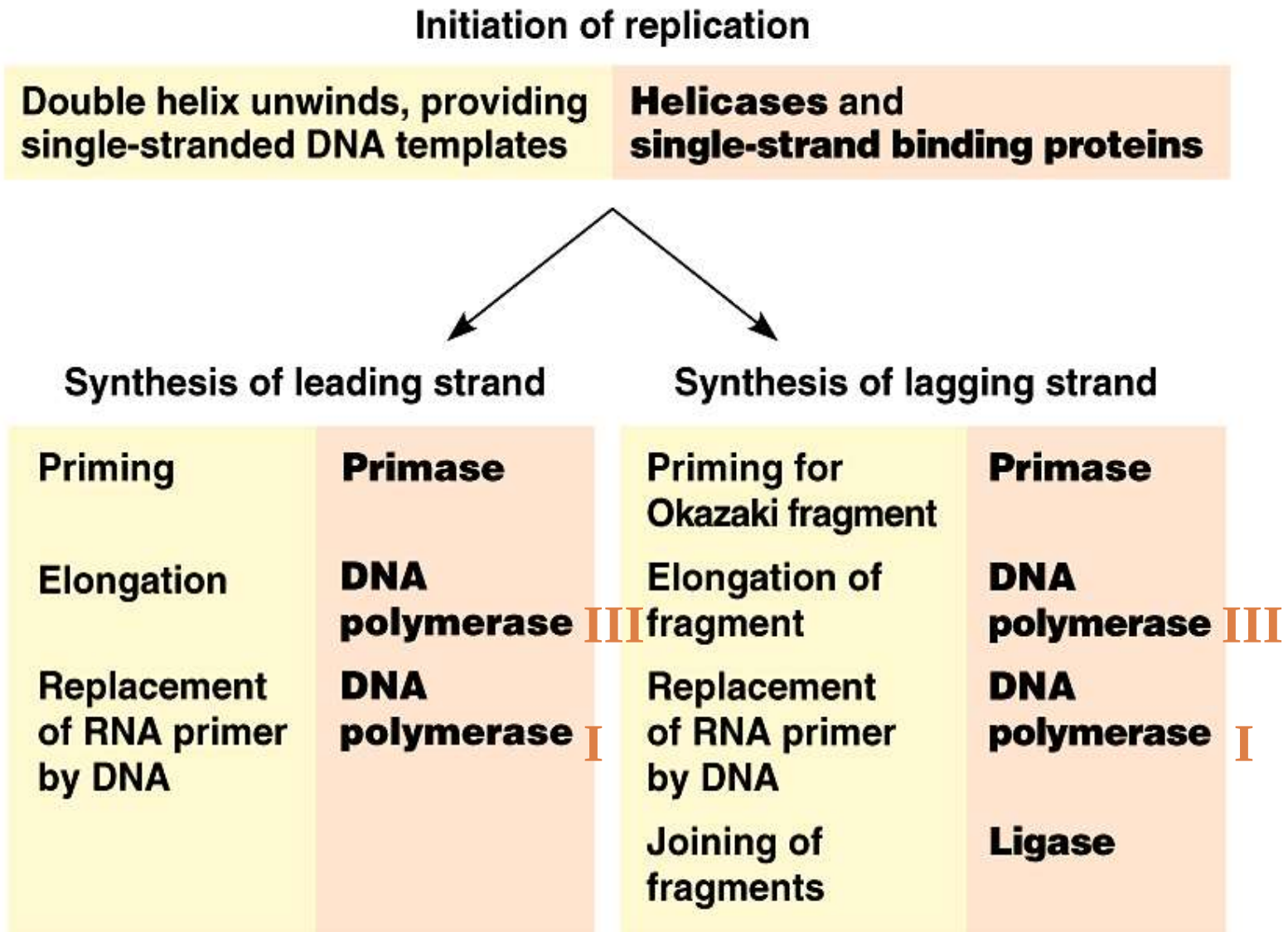


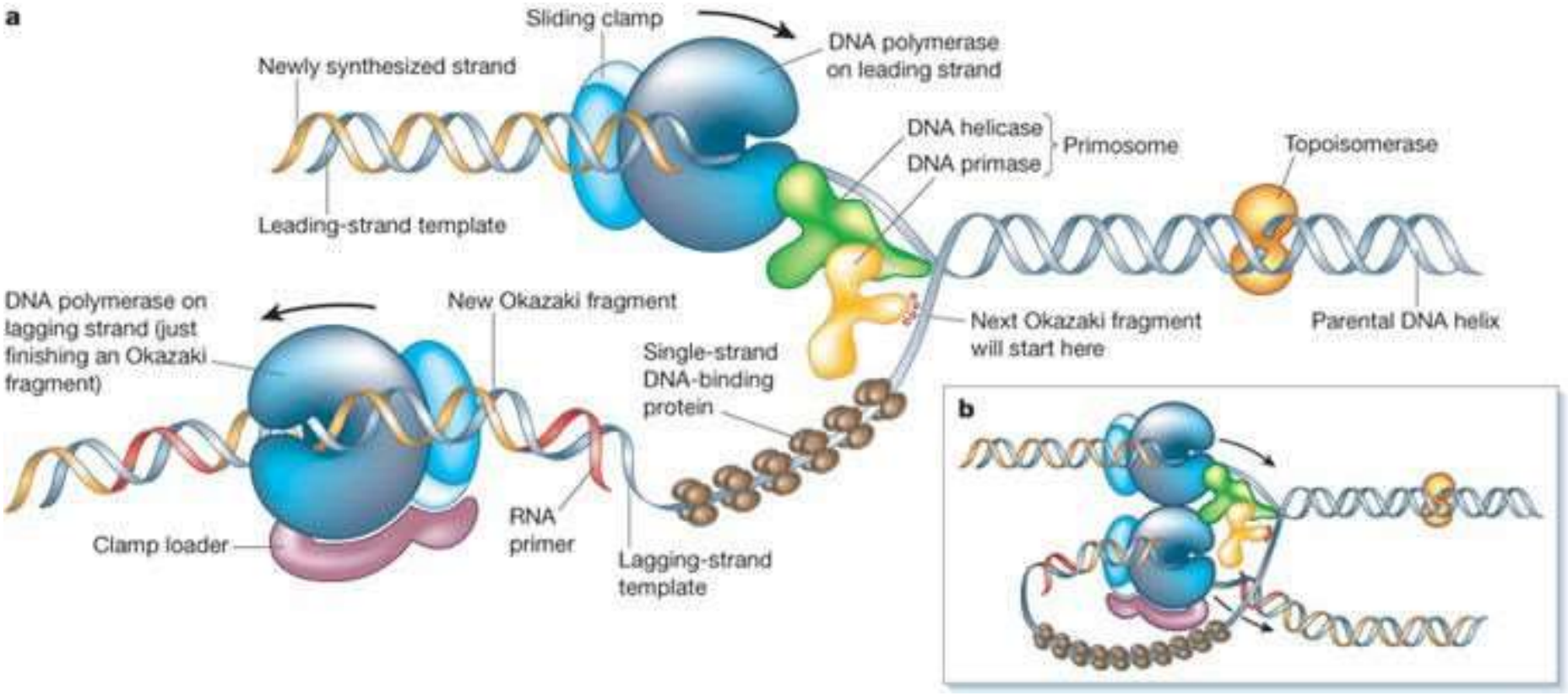
Fig. 16.15

The DNA Replication Complex

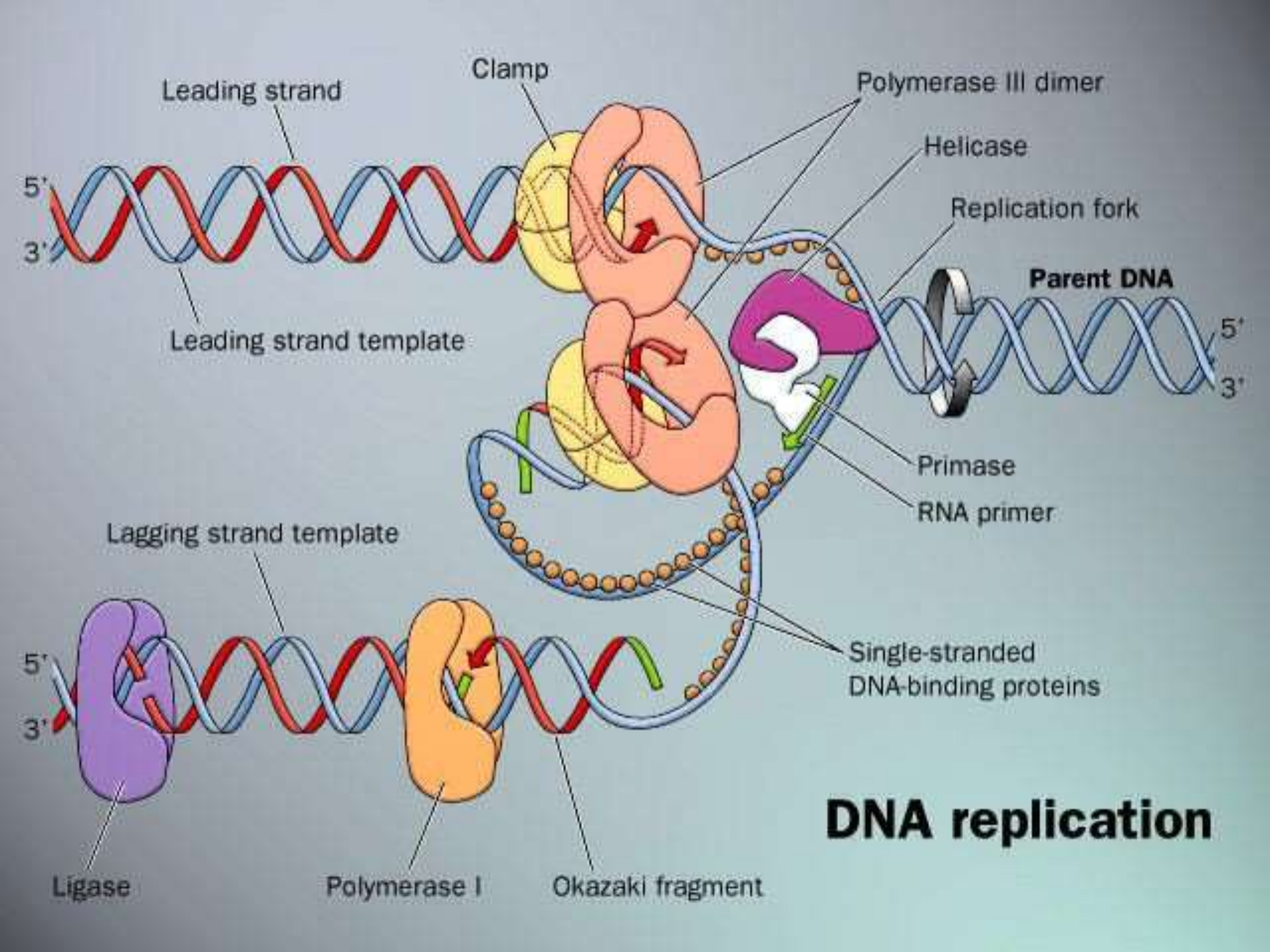
- Proteins involved in DNA replication form a single large complex anchored to the fibers in the nucleus.

- Stationary complex of DNA polymerase molecules pull in the parental DNA and produce newly made daughter DNA molecules.

DNA Replication Components



http://www.nature.com/nature/journal/v421/n6921/images/nature01407-i1.0.jpg

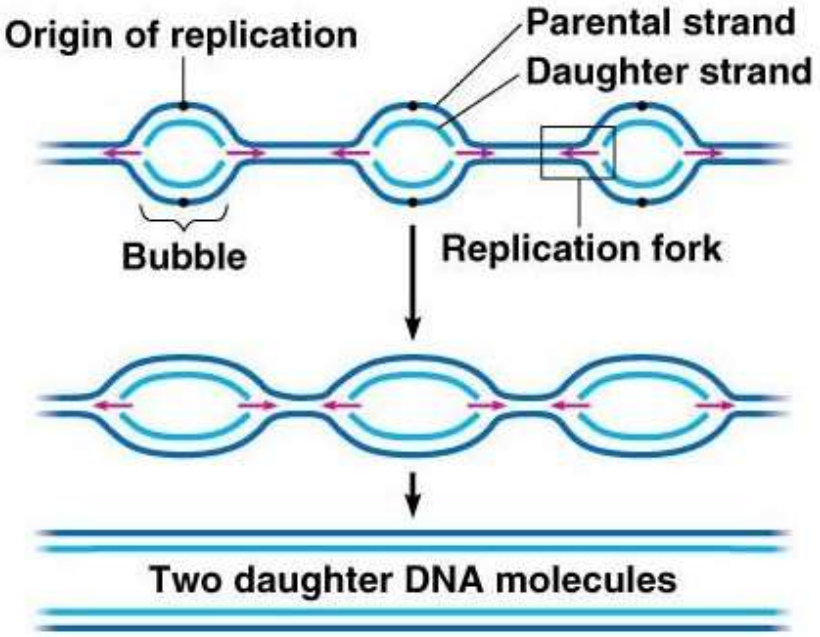

Leading strand
Clamp
Polymerase III dimer
Helicase
Replication fork
5'
3'
Parent DNA
Leading strand template
5'
3'
Primase
RNA primer
Lagging strand template
Single-stranded
DNA-binding proteins
5'
3'
Ligase
Polymerase I
Okazaki fragment
DNA replication

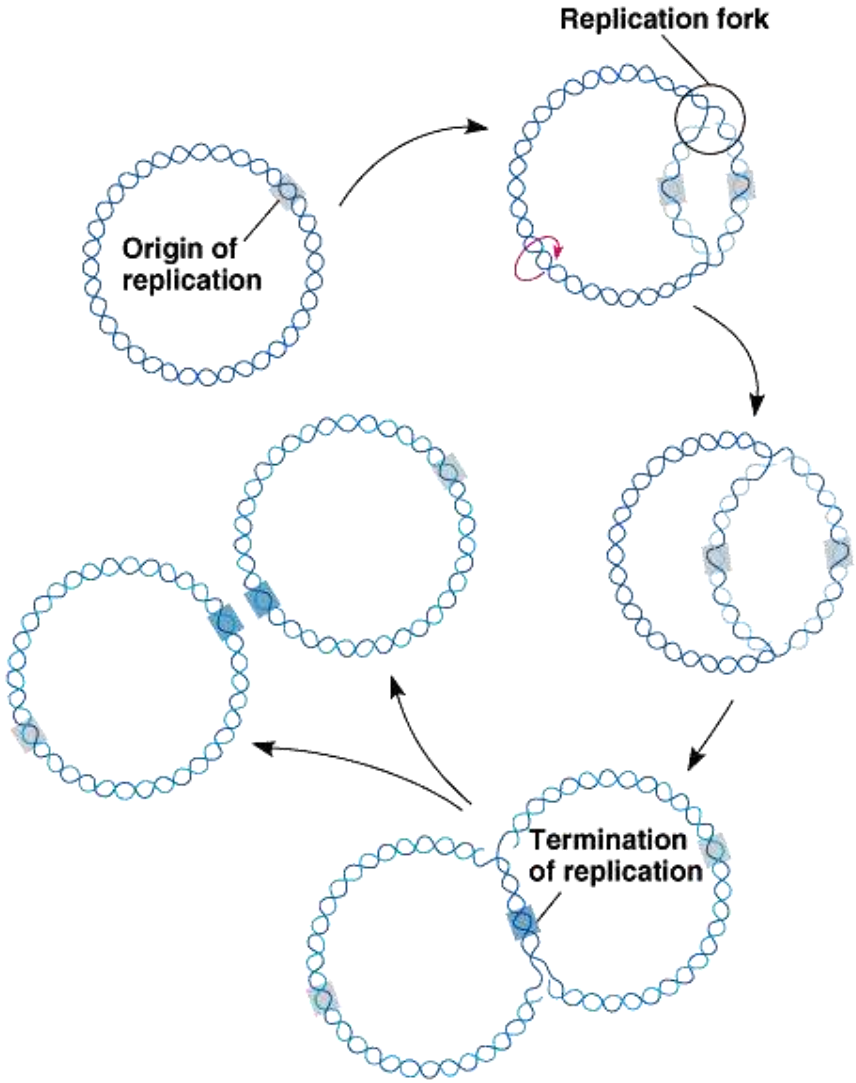
Video: 3D model visualization and explanation of replication

http://www.hhmi.org/biointeractive/dna/DNAi_replication_vo2.html

DNA replication: Termination

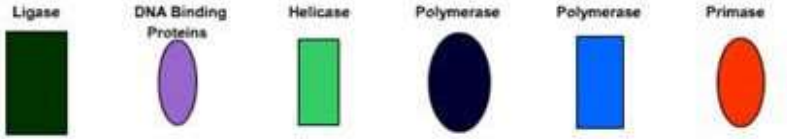
- DNA replication ends when:
  - Reach the end of the chromosome
  - Replication bubble / fork meets another replication bubble/ fork



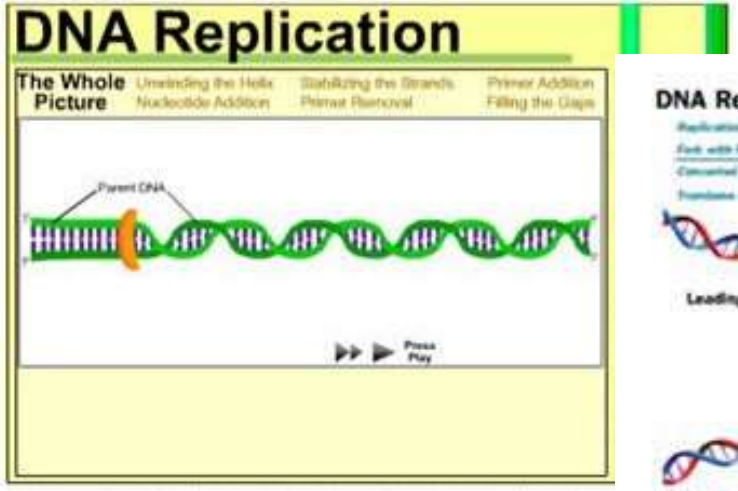


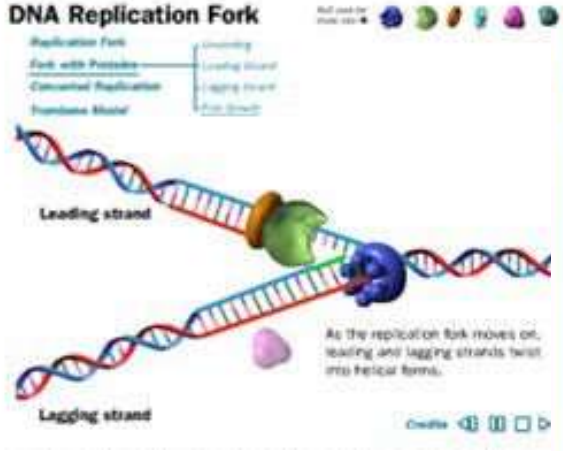
DNA Replication Tutorials

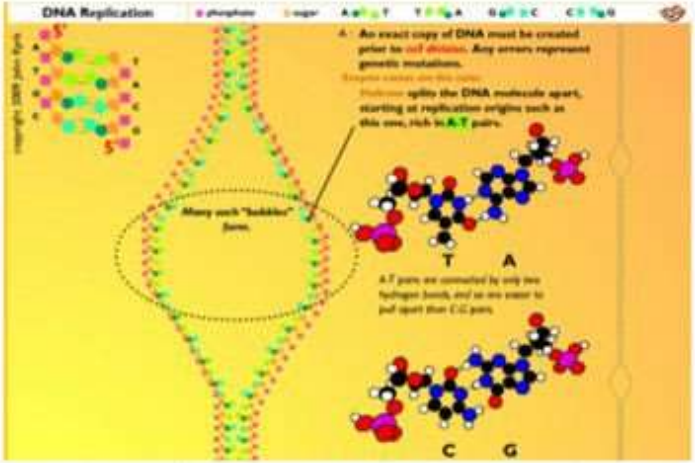
DNA Replication



- http://www.stolaf.edu/people/giannini/flashanimat/molgenetics/dna-rna2.swf
- http://www.wiley.com/legacy/college/boyer/0470003790/animations/replication/replication.swf
- http://www.wiley.com/college/pratt/0471393878/student/animations/dna_replication/index.html
- http://www.mcb.harvard.edu/Losick/images/TromboneFINALd.swf
- http://www.johnkyrk.com/DNAreplication.html







HW Questions

- Write out the point form summary of the steps in DNA replication. Include all the enzymes in the correct order.
- Since DNAP can only polymerize off an available 3′ end, where does DNAP I (the one that removes the RNA primer and replaces it with DNA) get that 3′ end from?
- Compare prokaryotic and eukaryotic replication and repair mechanisms by describing the differences. Use a comparative chart if possible.

3. DNA Repair: Proofreading

- Error Rate
- Types of Errors
- Exonuclease
- Endonuclease

Proofreading DNA

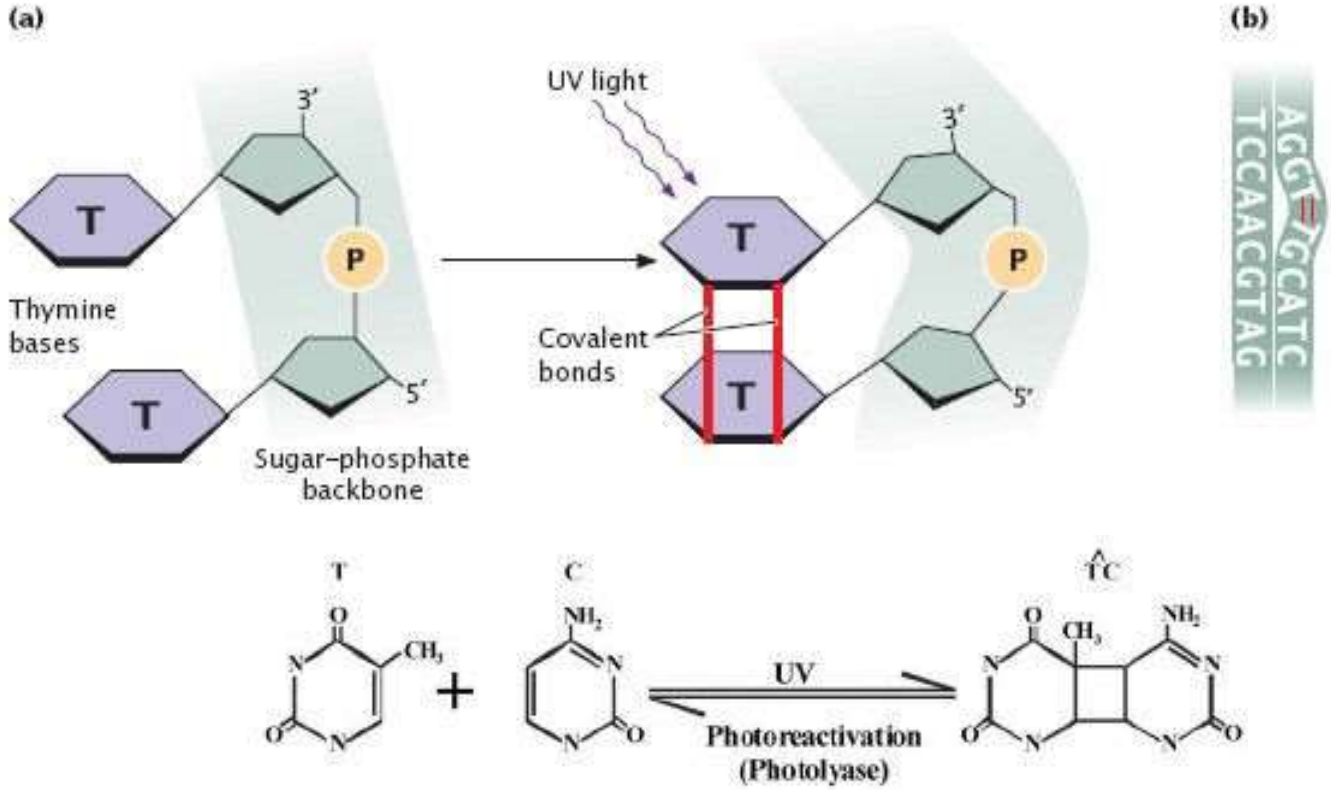
- Error rate
- Average human chromosome has 150,000,000 bp
- Initial pairing error: 1 in 10,000 bp = 15,000 errors per replication… that's a LOT
- Final error: 1 in 1,000,000,000 bp (1 in 10^9)
- Mechanisms in place to proofread errors as DNA is being replicated (exonuclease)
- Cell also continuously monitors and repairs DNA outside of replication (endonuclease)

Types of Errors in DNA

- **Mismatch** mutations:
- incorrectly paired bases
- one of the most common types of errors during replication
- Missing bases
- Fused bases

UV light

- A common cause of DNA damage
- Produces **pyrimidine dimers** (a type of fused base)



http://www.nature.com/scitable/nated/content/19185/pierce_17_24_FULL.jpg

Xeroderma Pigmentosum (XP)

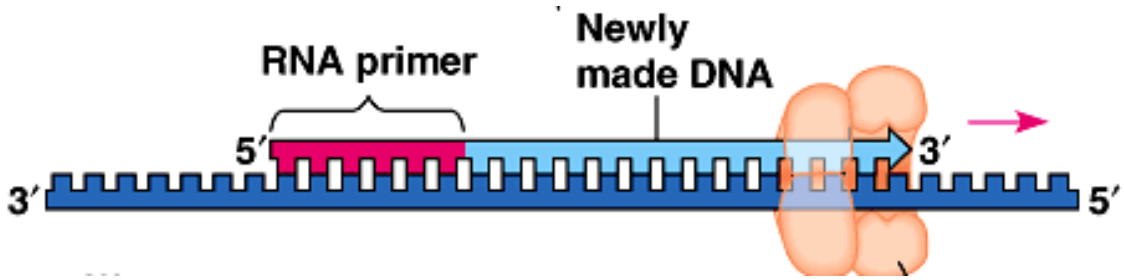
- A condition where individual is unable to repair damage caused by UV light
- Individuals may need to avoid sunlight completely ("children of the night")
- Leads to early skin cancer

Repair by Nuclease

- **Nuclease**: an enzyme that can break phosphodiester bonds in DNA thus **excising** out the nucleotide
- **Exonuclease**: binds to ends of nucleotide chain (5′ or 3′)
- **Endonuclease**: binds to the middle of a nucleotide chain

Exonuclease Proofreading

- Instantaneous repair:
- Occurs as the DNA is replicating
- Due to errors during elongation at the 3′ end
- DNAP III and DNAP I both have exonuclease activity



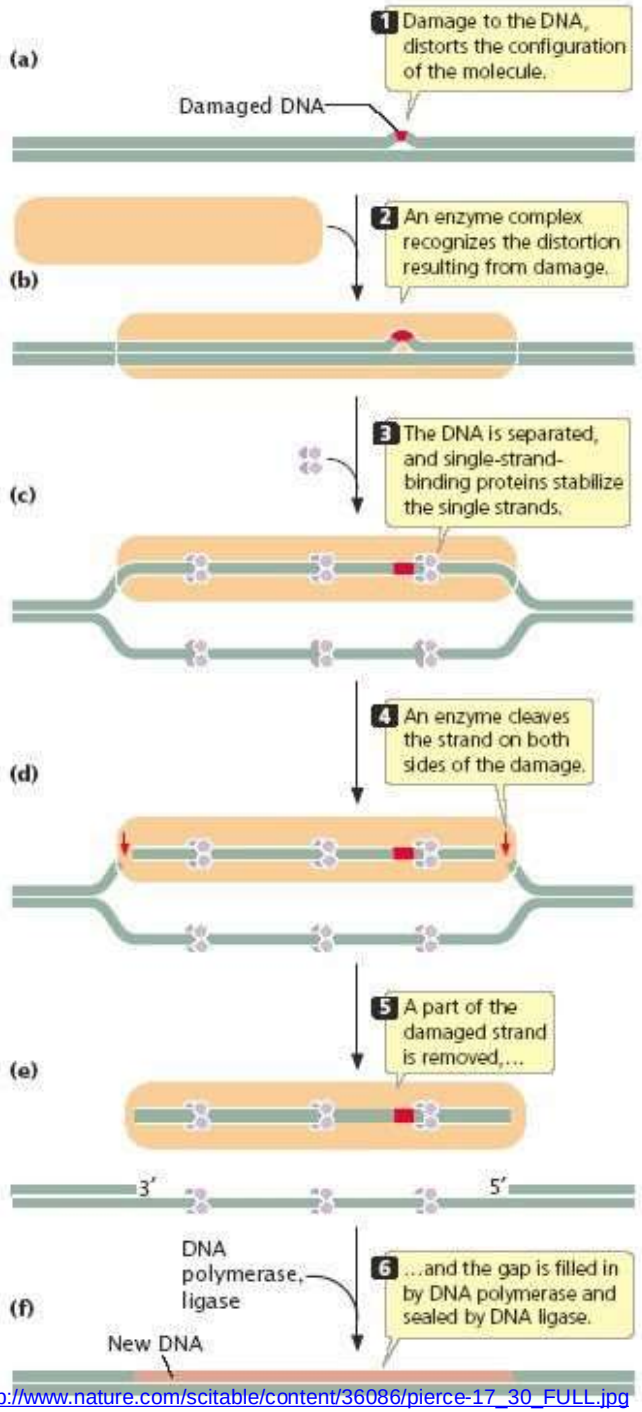
Exonuclease Proofreading

- Mechanism of repair:
- DNAP instantly recognize mismatches during replication
- hydrolyze the phosphodiester bond releasing the last nucleotide that was just added (exonuclease activity)
- replaces with the correct nucleotide (polymerase activity)

- Note: one enzyme (DNAP) does both the nuclease and polymerase function

Endonuclease Proofreading

- Repair often occurs after DNA is already replicated
- Mechanism of repair known as **nucleotide excision repair (NER)**



http://www.nature.com/scitable/content/36086/pierce-17_30_FULL.jpg

Endonuclease Proofreading: NER

- Endonuclease:
  - recognizes and binds to error
  - nicks the strand by breaking phosphodiester bonds
  - error is excised (removed)
- Polymerase: replaces the gap with the correct nucleotides
- Ligase: seals the nick

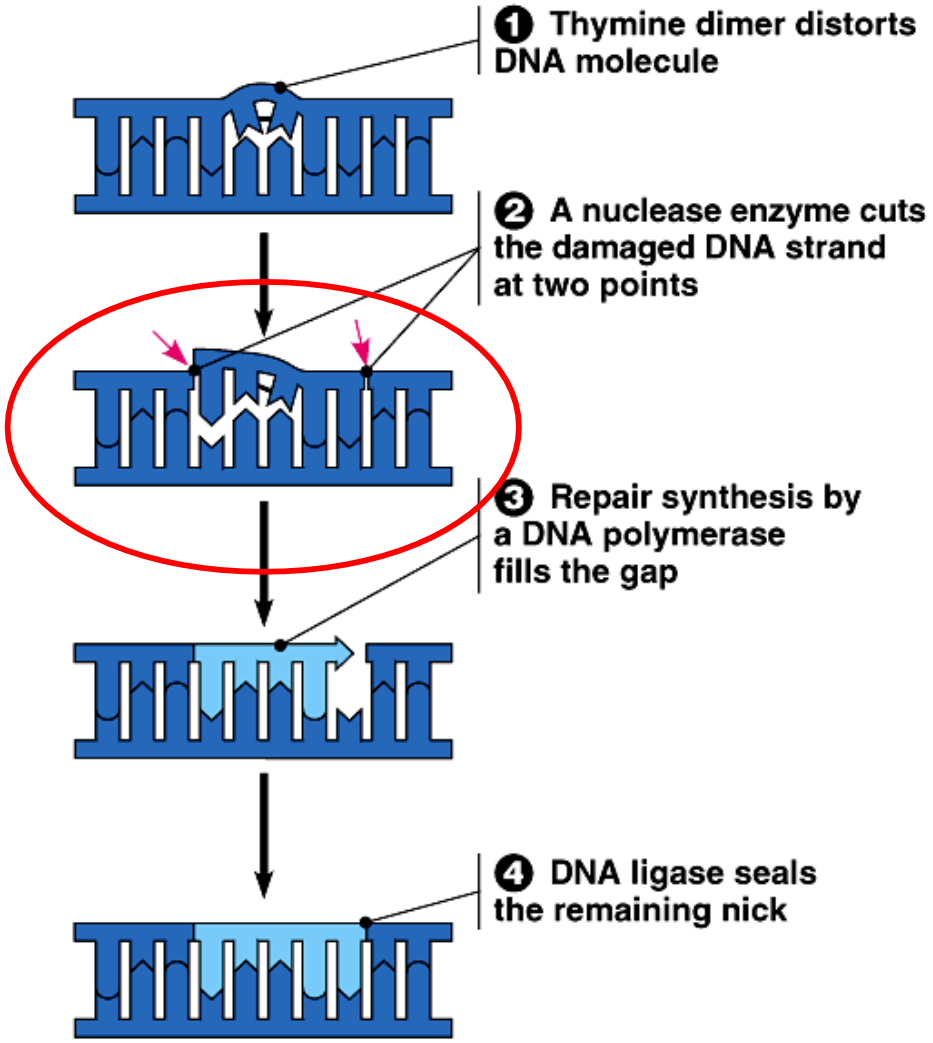
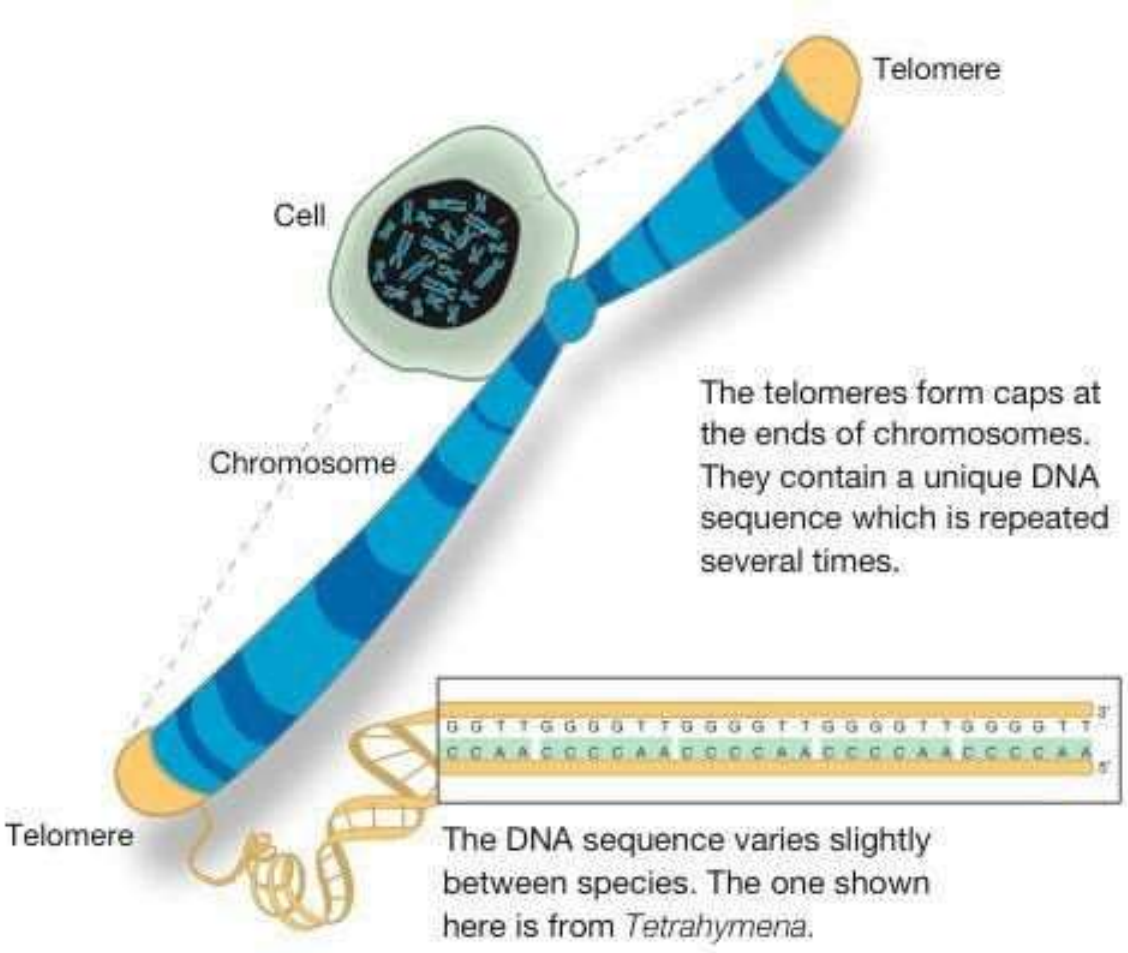


Fig. 16.17

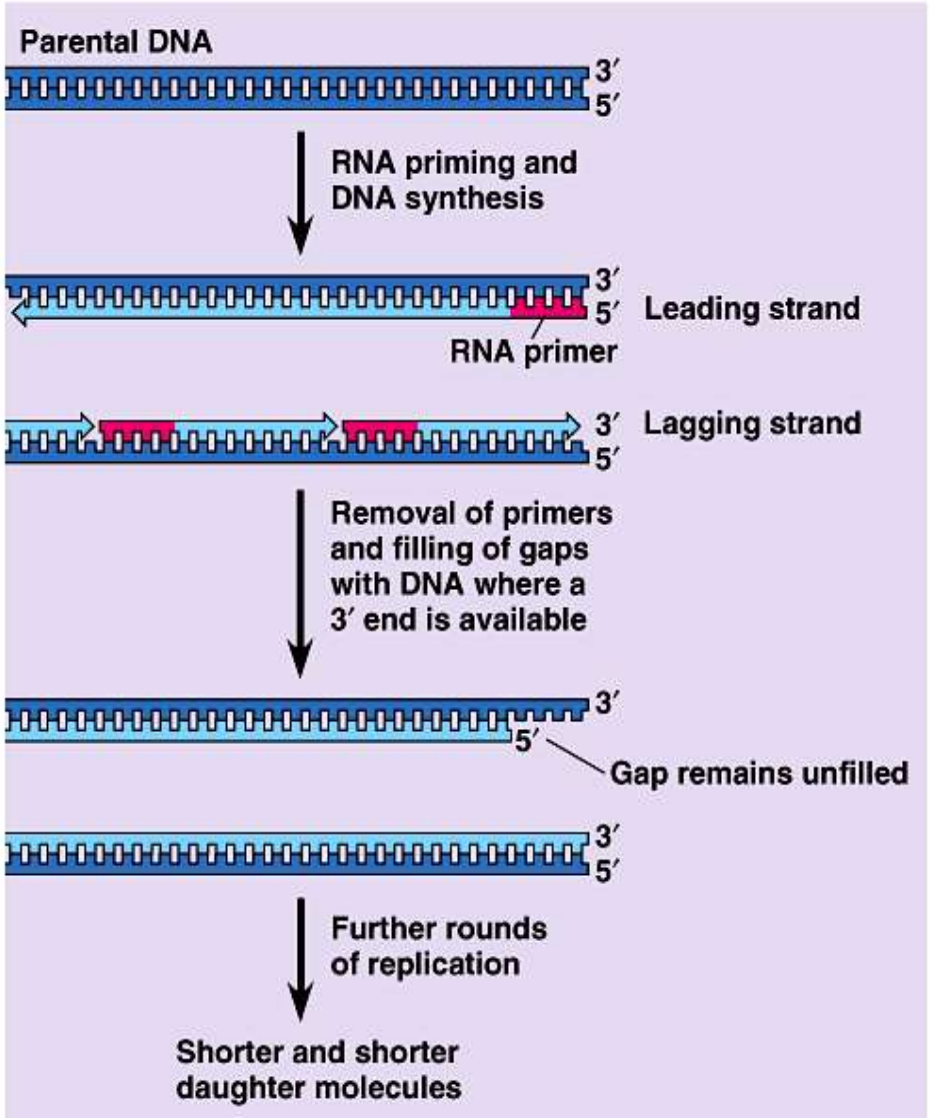
4. Telomeres

- Problem at the ends
- Aging
- Telomerase



http://images.sciencedaily.com/2009/10/091005110401-large.jpg

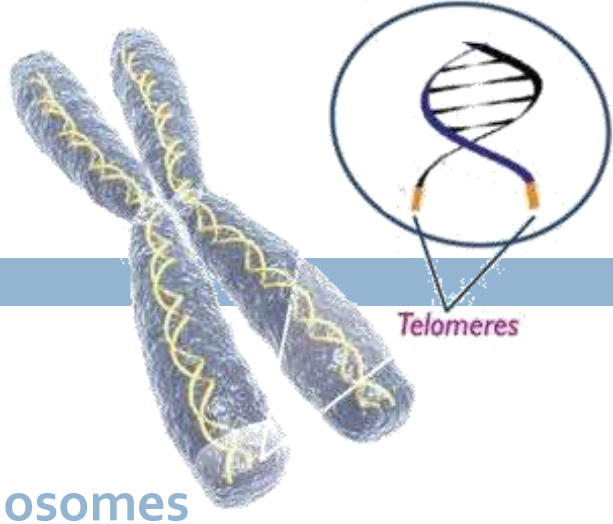
The problem with replication at the ends of linear DNA



- DNA gets progressively shorter with each round of replication
- Prokaryotes avoid problem by having circular DNA

Fig. 16.18

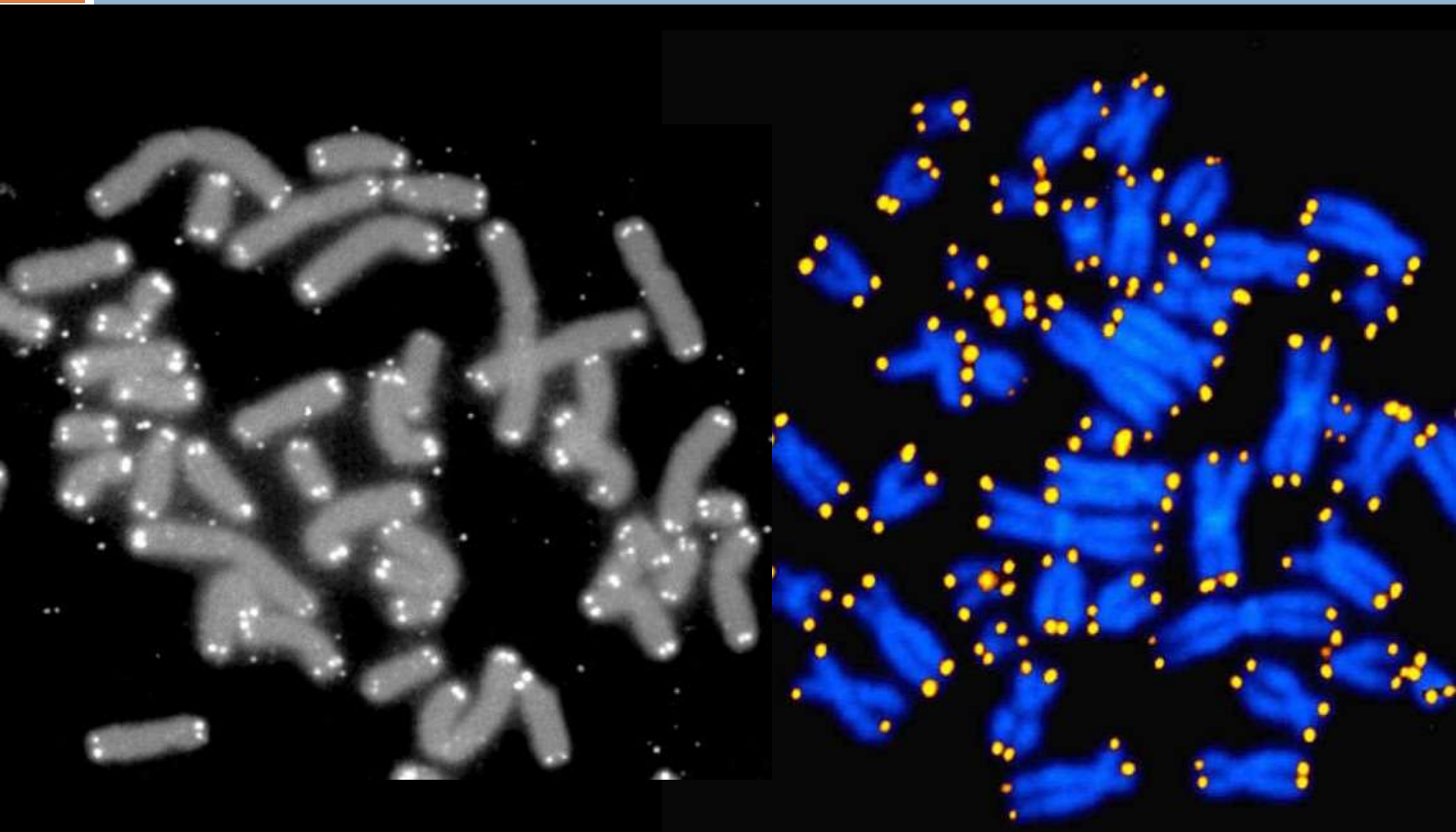
Telomere



- Structure:
  - DNA found at the **ends of eukaryotic chromosomes**
  - **Noncoding** (no genes)
  - Consists of **multiple repeats** of a short genetic sequence (humans: TTAGGG)
- Function:
  - protect chromosomes **from being eroded** through multiple rounds of DNA replication
  - Less about preserving genetic information and more about serving as a **protective cap** to prevent unwinding because
    - uncapping is sensed by cells
    - leads to cellular aging where cells stop growing and dividing (**senescence**) and/or programmed self-destruction (**apoptosis**)

http://www.dreva.com/shop/images/telomeres.jpg

Telomere



http://science.nasa.gov/media/medialibrary/2006/03/16/22mar_telomeres_resources/dapi.jpg https://media.npr.org/assets/img/2012/11/19/telomere-f87b8e7c2652d4f2d403da52beac222ce534b929-s6-c10.jpg

Telomere and Aging

- At **conception**, cells have telomeres that are about **15,000** bases long
- At **birth**, cells in the infant have telomeres that are

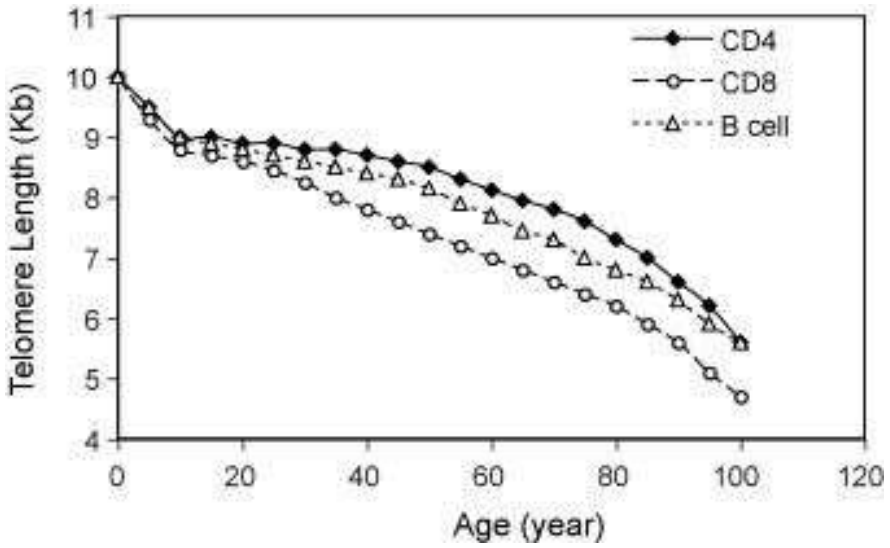
10,000 bases long

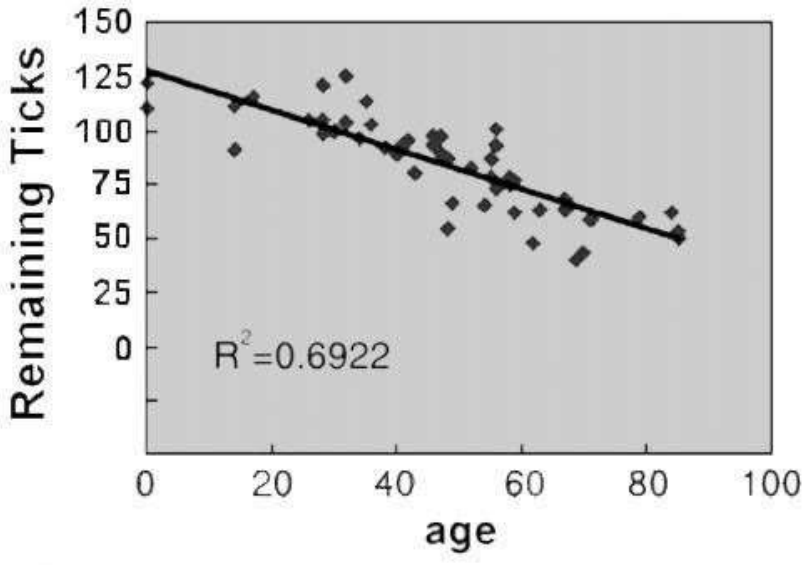
- Humans **die** from old age when telomere length shortens to about **5000** bases

You're born with the longest telomeres you'll ever have.

It only gets shorter with age.

Telomere and Aging





Weng, Nan-ping. "Telomere and adaptive immunity." *Mechanisms of Ageing and Development* 129.1-2 (2008): 60-66. Fig. 1. Model of telomere attrition in T and B cells with age.
Loss of telomere length is rapid during the first decade of life and decreases during most of adult life. At advanced age, the rate of telomere shortening may increase. The graph projects the telomere attrition in CD4, CD8, and B cells in vivo based on the cross-sectional analysis of telomere length in lymphocytes with age. Whether significantly shortened telomeres in advanced age cause declined function of lymphocytes will need further study.

http://www.sierrasci.com/telomere/index.html
The time remaining on this "telomere clock" can be measured from our blood cells. When such measurements are taken, a significant correlation is found between a person's age and the number of "ticks" remaining on the person's clock.

Telomere

Question:

- When are telomeres added to the chromosome when the length of the telomere is decreasing even at conception?

Answer:

- Telomeres added onto the ends of chromosomes **prior to birth, prior to conception, prior to fertilization**

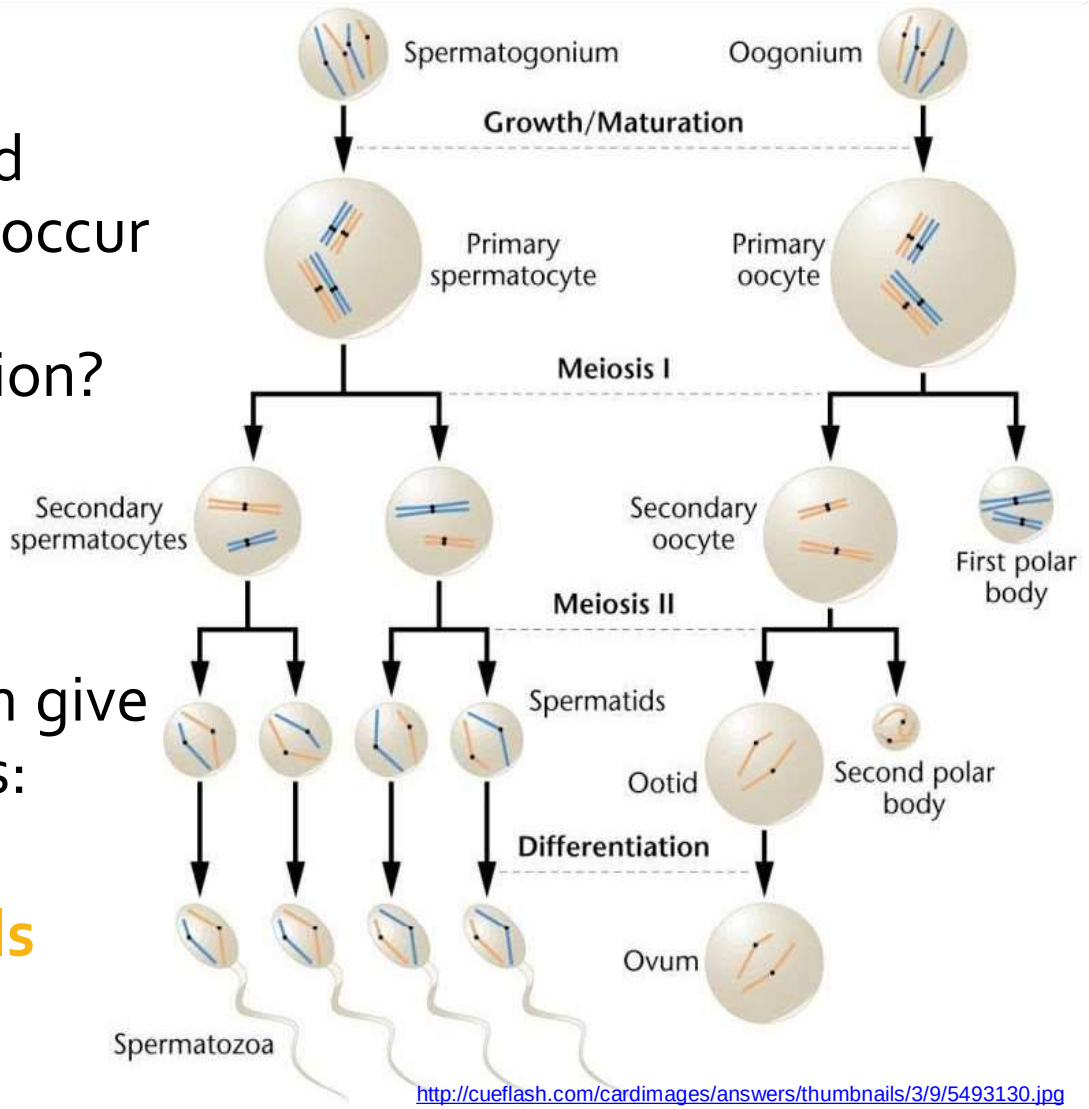
Telomere

Question:

- In what cell types would adding of the telomeres occur in if it happens before conception and fertilization?

Answer:

- In **germ line cells** which give rise to sex cells (gametes: sperm & egg)
- Also seen in **cancer cells**



http://cueflash.com/cardimages/answers/thumbnails/3/9/5493130.jpg

Telomerase

- Enzyme responsible for adding telomeres to chromosomes
- A **ribonucleoprotein** that extends the ends of chromosomes using the enzymatic action of **reverse transcriptase**

Telomerase Activity

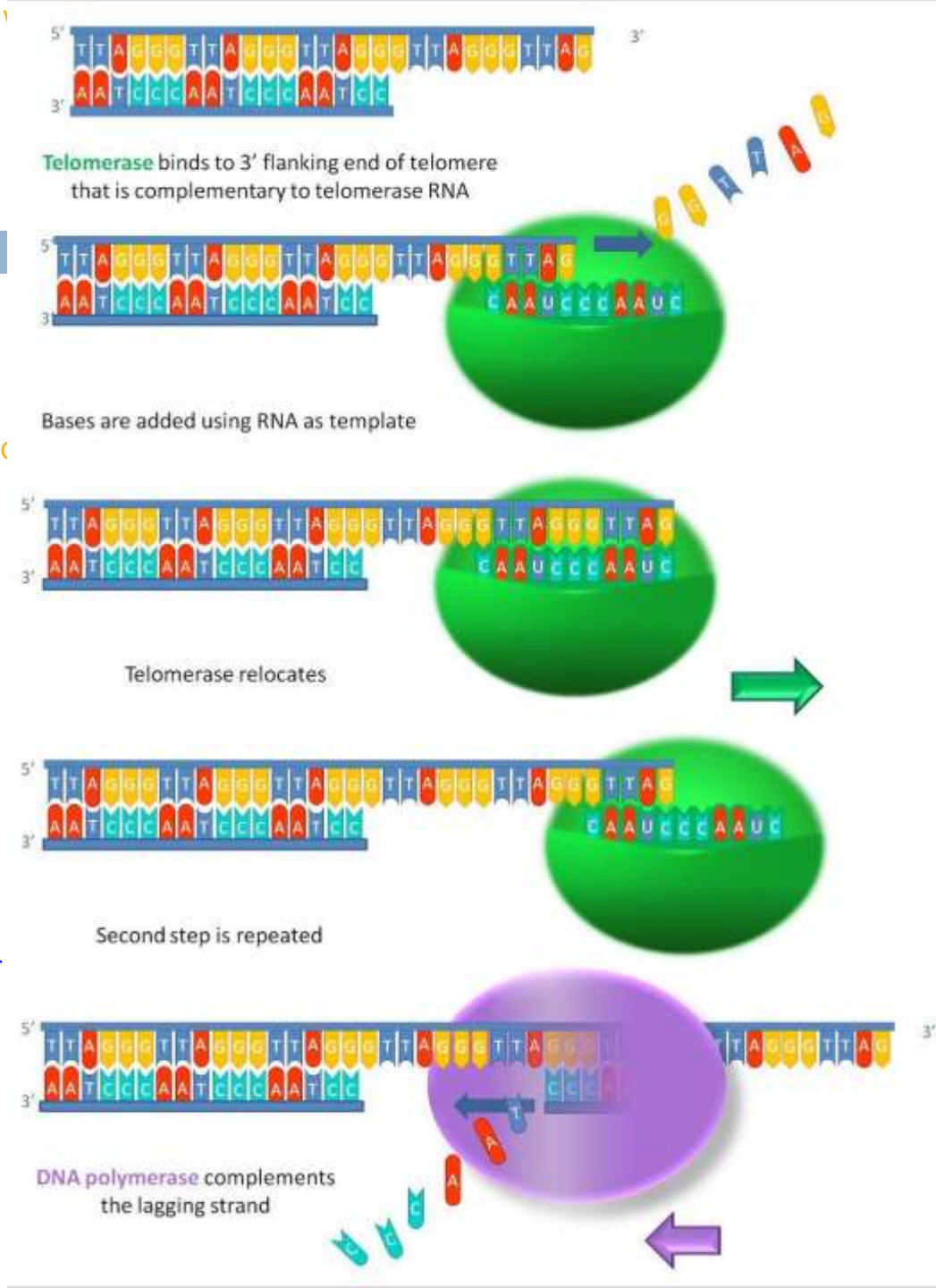
- Video clip:

http://www.youtube.com/watch?v=AJNoTm

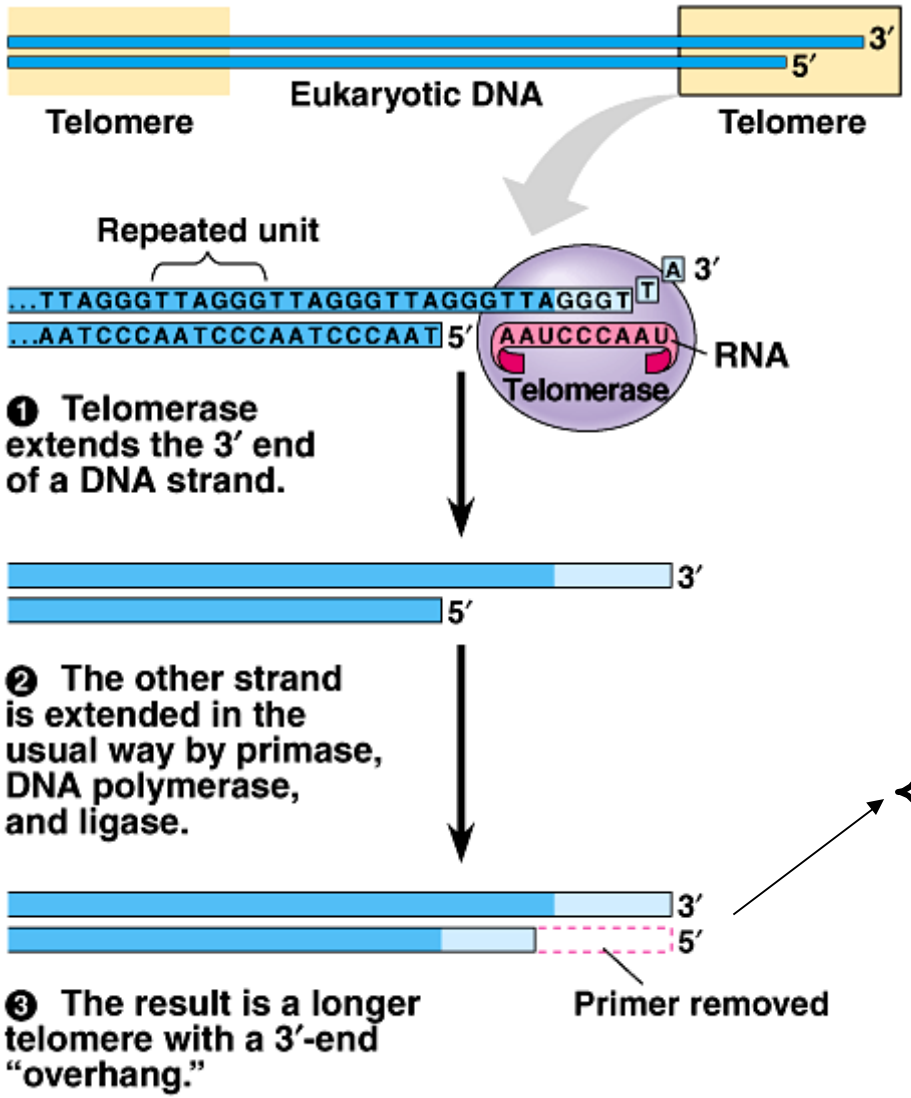
- Comprehensive series of lectures on telomeres and telomerase by Elizabeth Blackburn (discovered the molecular nature of telomeres and telomerase):

http://www.ibioseminars.org/lectures/cell-bi

- Lecture 1 – Telomeres & Telomerase (48:27)
- Lecture 2 – Telomeres & Telomerase in Human Stem Cells & Cancer (26:58)
- Lecture 3 – Stress, Telomeres & Telomerase in Humans (45:58)



Telomerase Activity



- Binds to 3′ end (parental strand)
- Extends the chain by reverse transcribing off its internal RNA template (repeats)
- Primase adds primer
- DNAP α elongates
- Ligase seals the chain

Fig. 16.19

Telomerase

- **Ribonucleoprotein**:
- an enzyme that contains protein and RNA
- human telomerase has an RNA component that contains the internal sequence **AAUCCC**
- **Reverse transcriptase**:
- synthesis of complementary DNA from an RNA template
- uses its internal RNA sequence as a template to make a complementary DNA strand with sequence **TTAGGG**
- extends the 3′ end of the chromosome

Telomerase and Aging

- Telomerase is only found in certain cells
- Germ line cells
- Cancer cells
- Cells that have telomerase live for a longer period of time
- The lack of telomerase in most cells may explain why cells have a finite lifespan
- Example: DNA of dividing somatic cells (non-sex cells) tend to be shorter in older individuals

Telomerase and Aging

- Why would germ line cells need telomerase?
- What would happen if germ line cells don't have telomerase?

Scientist suspect that one of the reasons why Dolly (the first mammal cloned from an adult somatic cell) didn't live as long was because its telomeres were the length of the adult that it was cloned from (as opposed to being as long as a typical newly born sheep)

http://1.bp.blogspot.com/-E1HNtngaNio/T_uldNBEfeI/AAAAAAAAA0c/dRTiYI12efM/s1600/Baby+Daddy+(final).jpg

Videos: Telomere and Aging

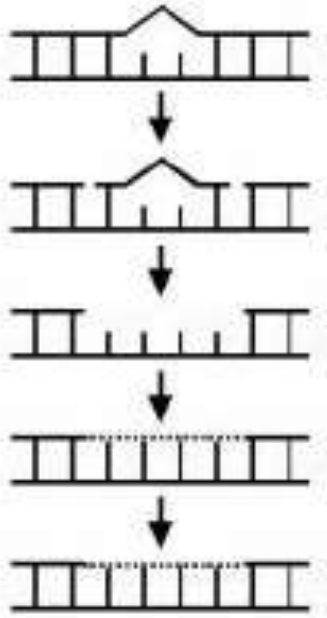
- http://www.youtube.com/watch?v=IzinjLhZXpA (BBC TV "Don't grow old", 3:41)
- http://www.youtube.com/watch?v=xI70O69EZY8 (Today Show "How to live to 100", 5:59)
- http://www.youtube.com/watch?v=m3qqUy88odQ

(Isagenix, 11:08)

- http://www.youtube.com/watch?v=lBngws_cWho (TedMed, 12:42)
- http://www.youtube.com/watch?v=-bmMv6dcsgE

(Independent Pharmacy Business Growth Conference, February 23, 2012 in Orlando, FL, 1:44:06)

HW Questions



- Refer to the diagram on the right.
  - Name the process shown
  - Describe what is happening in each step.
  - Name the enzymes involved in each step.
- Why does telomerase extend the 3′ end of the longer DNA strand?
- Why is it important that germ line cells have telomerase when all other cells in our body do not?
- What characteristic of cancer cells require them to have telomerase activity? Explain.
- Explain why artificially adding telomerase to a cell in the body won't make that cell cancerous?

DNA Replication and Repair Machinery

- Helicase
- SSBPs
- Topoisomerase (gyrase)
- Primase (a RNAP)
- DNAP III
- DNAP I
- DNA ligase
- Nuclease
- Telomerase