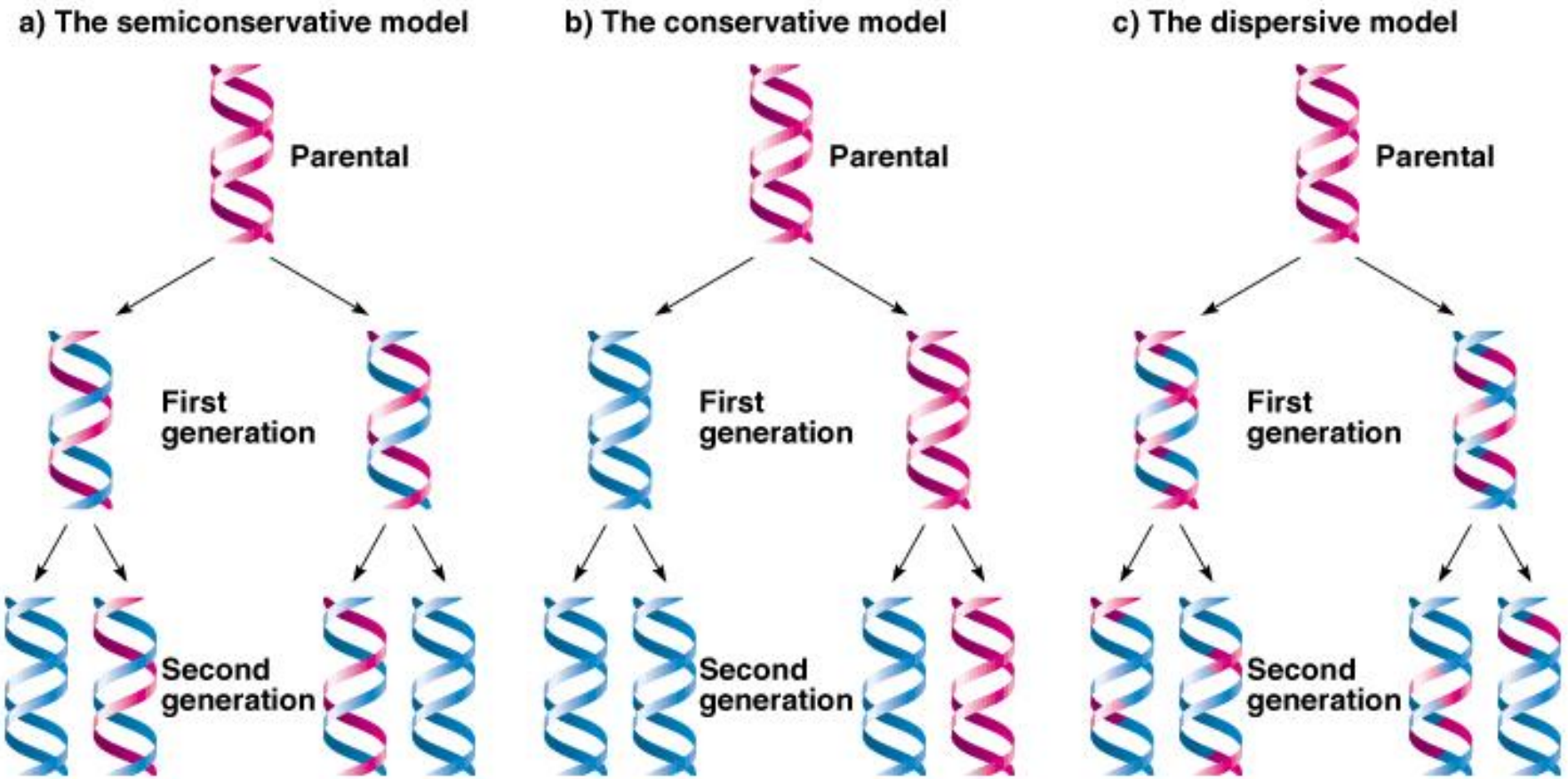
DNA Replication

Shamiyath A.
Assistant Professor
Dept. of Zoology
MES Keveeyam College,
Valanchery

Chapter 3: DNA Replication

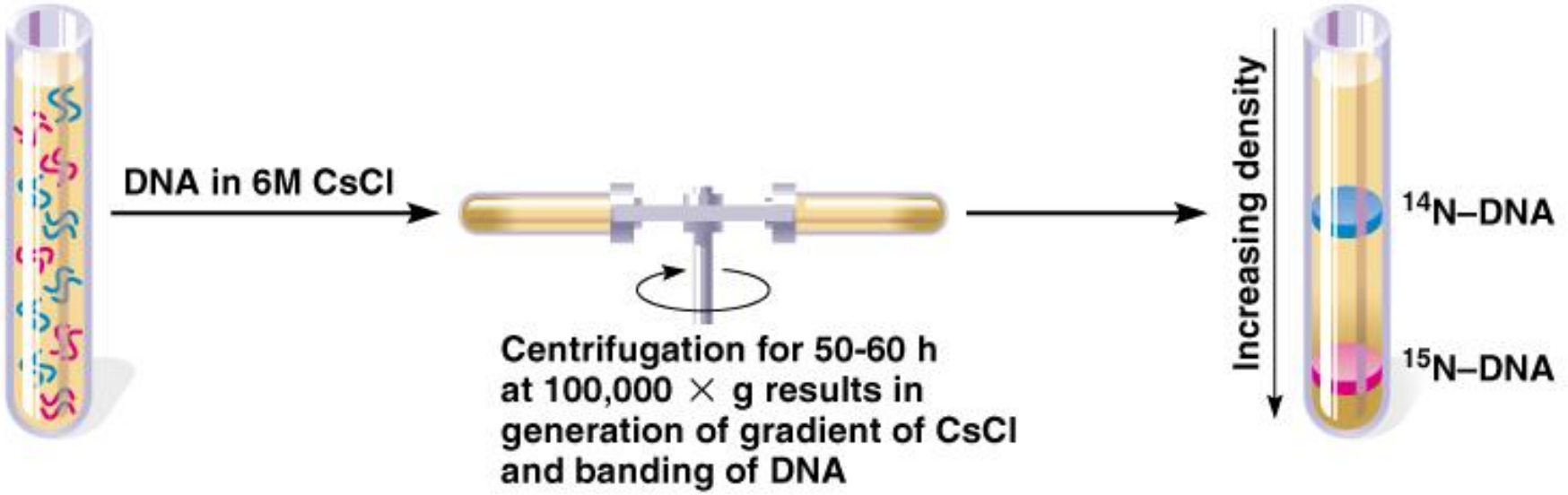
1. **Models of DNA replication: Meselson-Stahl Experiment**
2. **DNA synthesis and elongation**
3. **DNA polymerases**
4. **Origin and initiation of DNA replication**
5. **Prokaryote/eukaryote models (circular/linear chromosomes)**
6. **Telomere replication**
7. **Histone/chromatin assembly**

Alternative models of DNA replication (Fig 3.1):



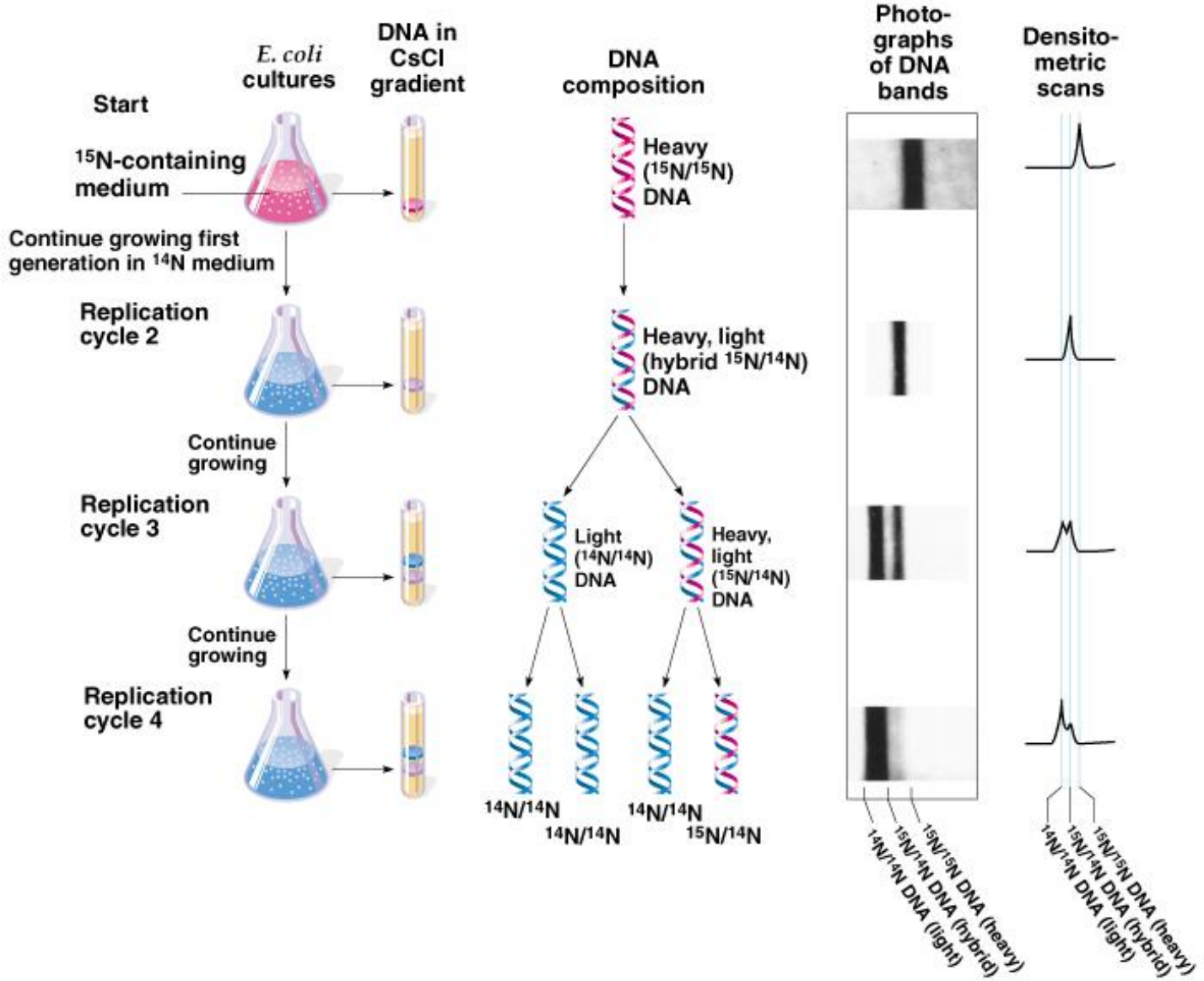
1958: Matthew Meselson & Frank Stahl's Experiment

Equilibrium density gradient centrifugation (Box 3.1)

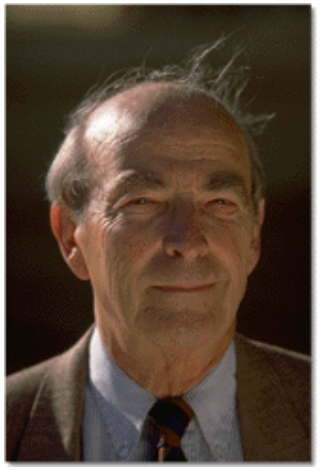


1958: Matthew Meselson & Frank Stahl's Experiment

Semiconservative model of DNA replication (Fig. 3.2)



1955: Arthur Kornberg



Worked with *E. coli*.
Discovered the mechanisms of DNA synthesis.

Four components are required:

1. **dNTPs: dATP, dTTP, dGTP, dCTP (deoxyribonucleoside 5'-triphosphates) (sugar-base + 3 phosphates)**

2. **DNA template**

3. **DNA polymerase (*Kornberg enzyme*)**

4. **Mg^{2+} (optimizes DNA polymerase activity)**

1959: Arthur Kornberg (Stanford University) & Severo Ochoa (NYU)

Three main features of the DNA synthesis reaction:

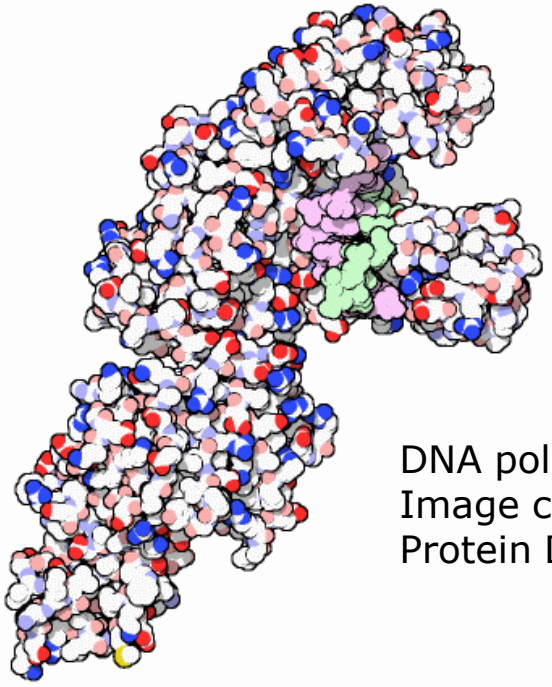
1. **DNA polymerase I catalyzes formation of phosphodiester bond between 3'-OH of the deoxyribose (on the last nucleotide) and the 5'-phosphate of the dNTP.**

- **Energy for this reaction is derived from the release of two of the three phosphates of the dNTP.**

2. **DNA polymerase "finds" the correct complementary dNTP at each step in the lengthening process.**

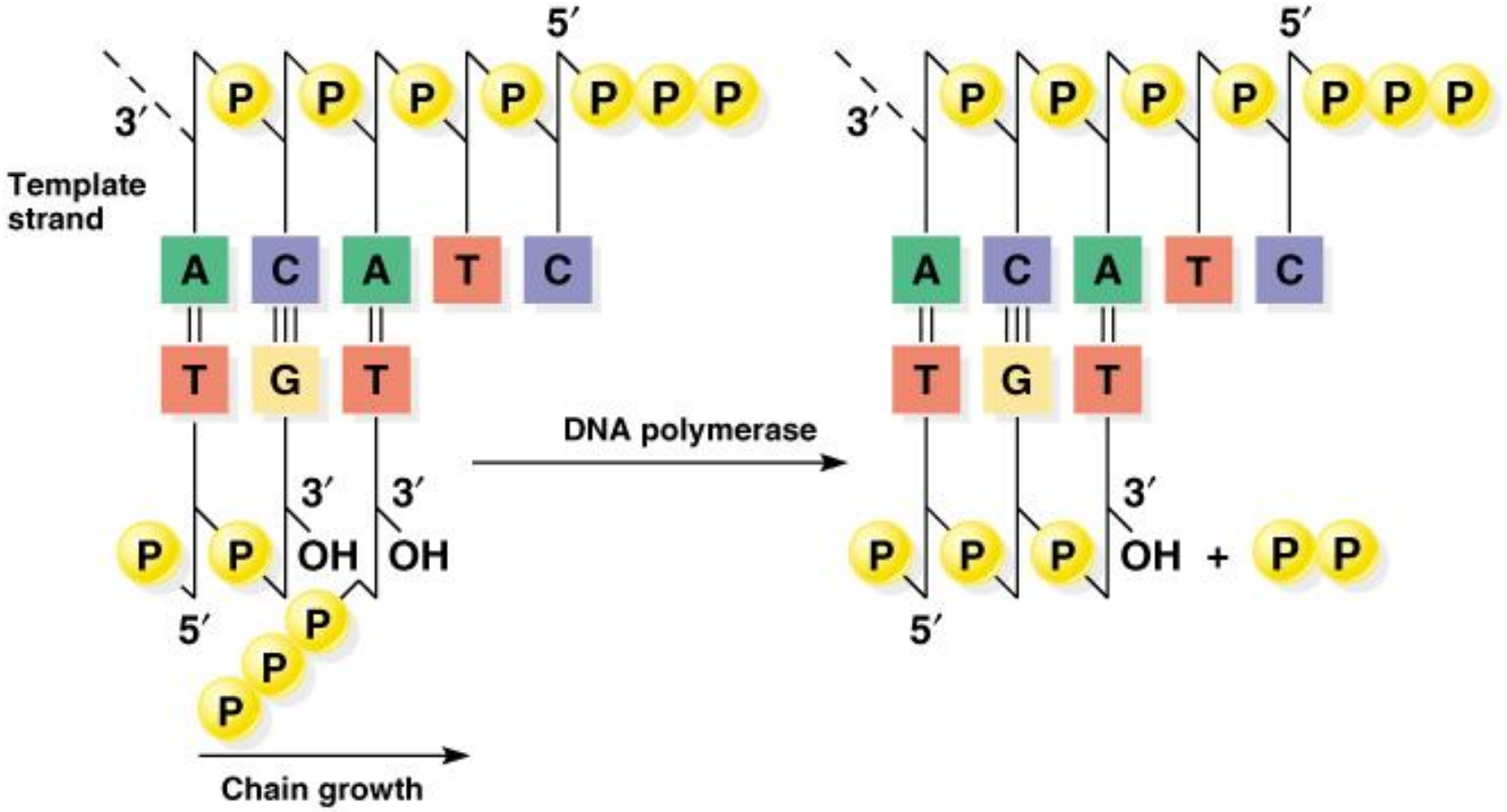
- **rate ≤ 800 dNTPs/second**
- **low error rate**

3. **Direction of synthesis is 5' to 3'**



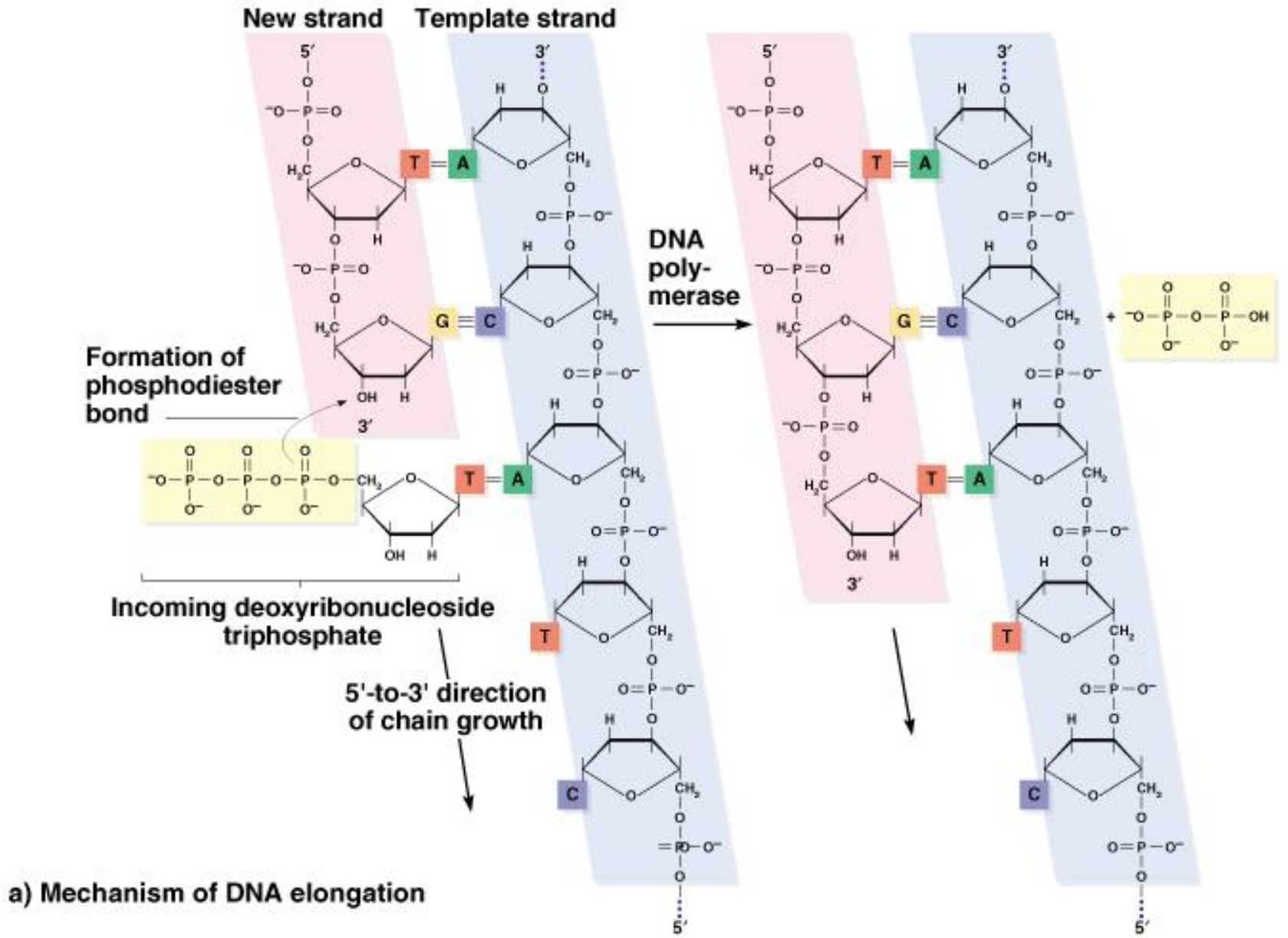
DNA polymerase
Image credit:
Protein Data Bank

DNA elongation (Fig. 3.3b):



b) Shorthand notation

DNA elongation (Fig. 3.3a):



In prokaryotes, there are three main types of DNA polymerase

Polymerase	Polymerization (5'-3')	Exonuclease (3'-5')	Exonuclease (5'-3')	#Copies
I	Yes	Yes	Yes	400
II	Yes	Yes	No	?
III	Yes	Yes	No	10-20

- **3' to 5' exonuclease activity = ability to remove nucleotides from the 3' end of the chain**
  - **Important proofreading ability**
  - **Without proofreading error rate (mutation rate) is 1×10^{-6}**
  - **With proofreading error rate is 1×10^{-9} (1000-fold decrease)**
- **5' to 3' exonuclease activity functions in DNA replication & repair.**

Eukaryotic enzymes:

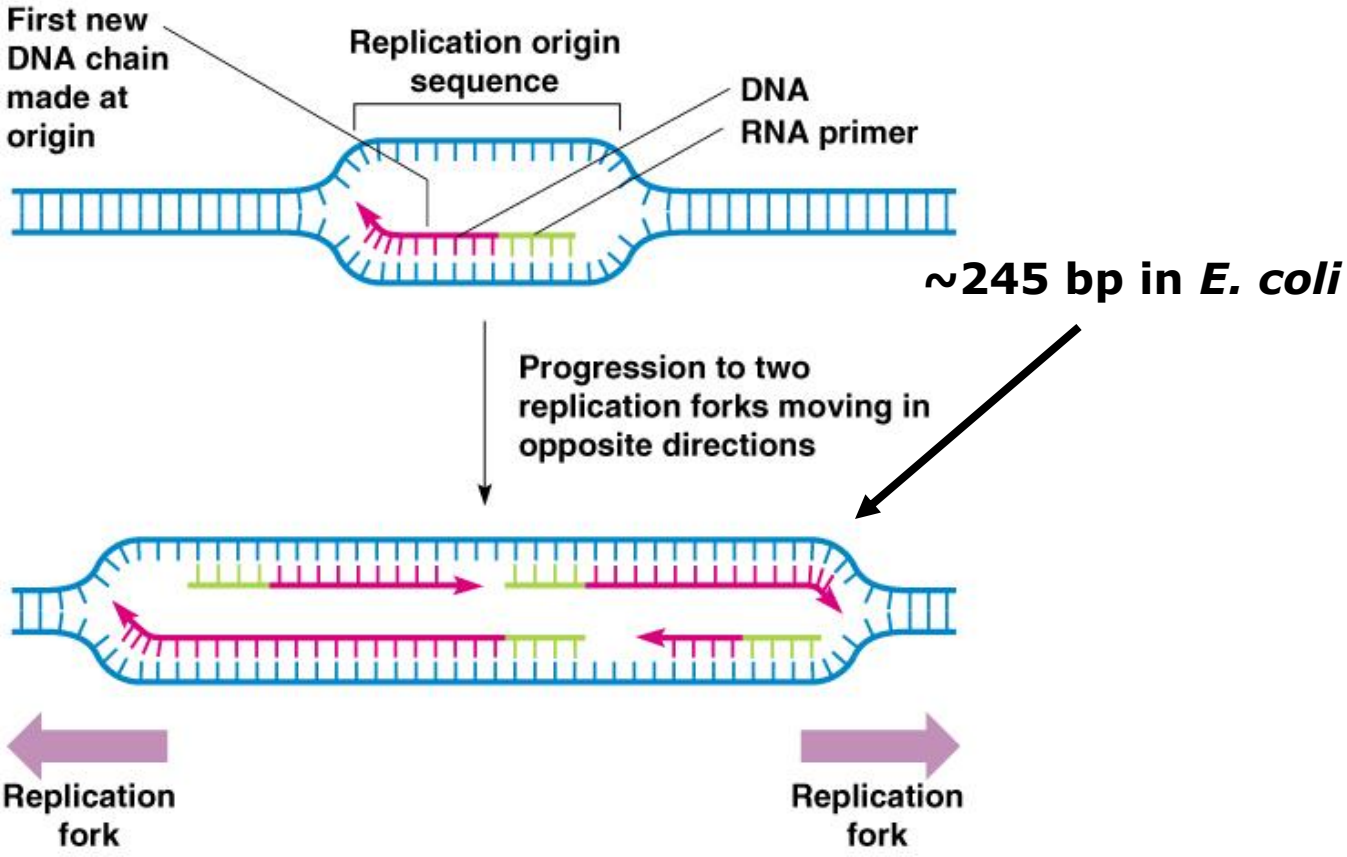
Five common DNA polymerases from mammals.

1. **Polymerase α (alpha): nuclear, DNA replication, no proofreading**
2. **Polymerase β (beta): nuclear, DNA repair, no proofreading**
3. **Polymerase γ (gamma): mitochondria, DNA repl., proofreading**
4. **Polymerase δ (delta): nuclear, DNA replication, proofreading**
5. **Polymerase ε (epsilon): nuclear, DNA repair (?), proofreading**

- **Different polymerases for the nucleus and mtDNA**
- **Some polymerases proofread; others do not.**
- **Some polymerases used for replication; others for repair.**
- **Polymerases vary by species.**

Origin of replication (e.g., the prokaryote example):

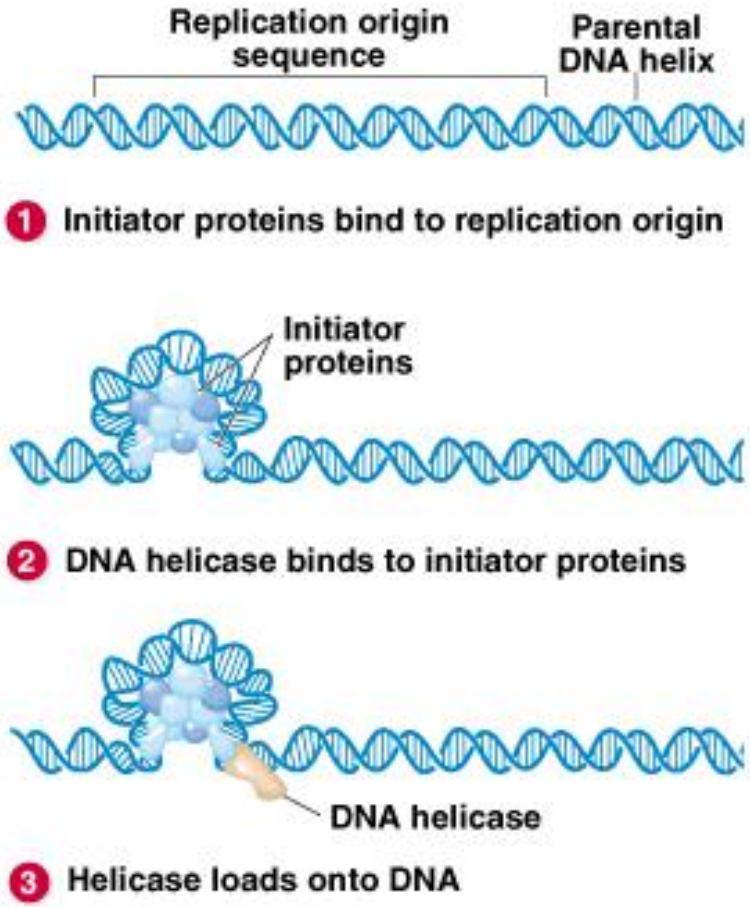
- ✓ **Begins with double-helix denaturing into single-strands thus exposing the bases.**
- ✓ **Exposes a replication bubble from which replication proceeds in both directions.**



Initiation of replication, major elements:

- ✓ **Segments of single-stranded DNA are called template strands.**
- ✓ **Gyrase (a type of topoisomerase) relaxes the supercoiled DNA.**
- ✓ **Initiator proteins and DNA helicase binds to the DNA at the replication fork and untwist the DNA using energy derived from ATP (adenosine triphosphate).**
  (Hydrolysis of ATP causes a shape change in DNA helicase)
- ✓ **DNA primase next binds to helicase producing a complex called a primosome (primase is required for synthesis),**
- ✓ **Primase synthesizes a short RNA primer of 10-12 nucleotides, to which DNA polymerase III adds nucleotides.**
- ✓ **Polymerase III adds nucleotides 5' to 3' on both strands beginning at the RNA primer.**
- ✓ **The RNA primer is removed and replaced with DNA by polymerase I, and the gap is sealed with DNA ligase.**
- ✓ **Single-stranded DNA-binding (SSB) proteins (>200) stabilize the single-stranded template DNA during the process.**

Model of replication in *E. coli*



DNA replication is continuous on the leading strand and semidiscontinuous on the lagging strand:

Unwinding of any single DNA replication fork proceeds in one direction.

The two DNA strands are of opposite polarity, and DNA polymerases only synthesize DNA 5' to 3'.

Solution: DNA is made in opposite directions on each template.

- **Leading strand** — **synthesized 5' to 3' in the direction of the replication fork movement.**

  continuous

  requires a single RNA primer

- **Lagging strand** — **synthesized 5' to 3' in the opposite direction.**

  semidiscontinuous (i.e., not continuous)

  requires many RNA primers , DNA is synthesized in short fragments.

Supercoiled DNA relaxed by gyrase & unwound by helicase + proteins:

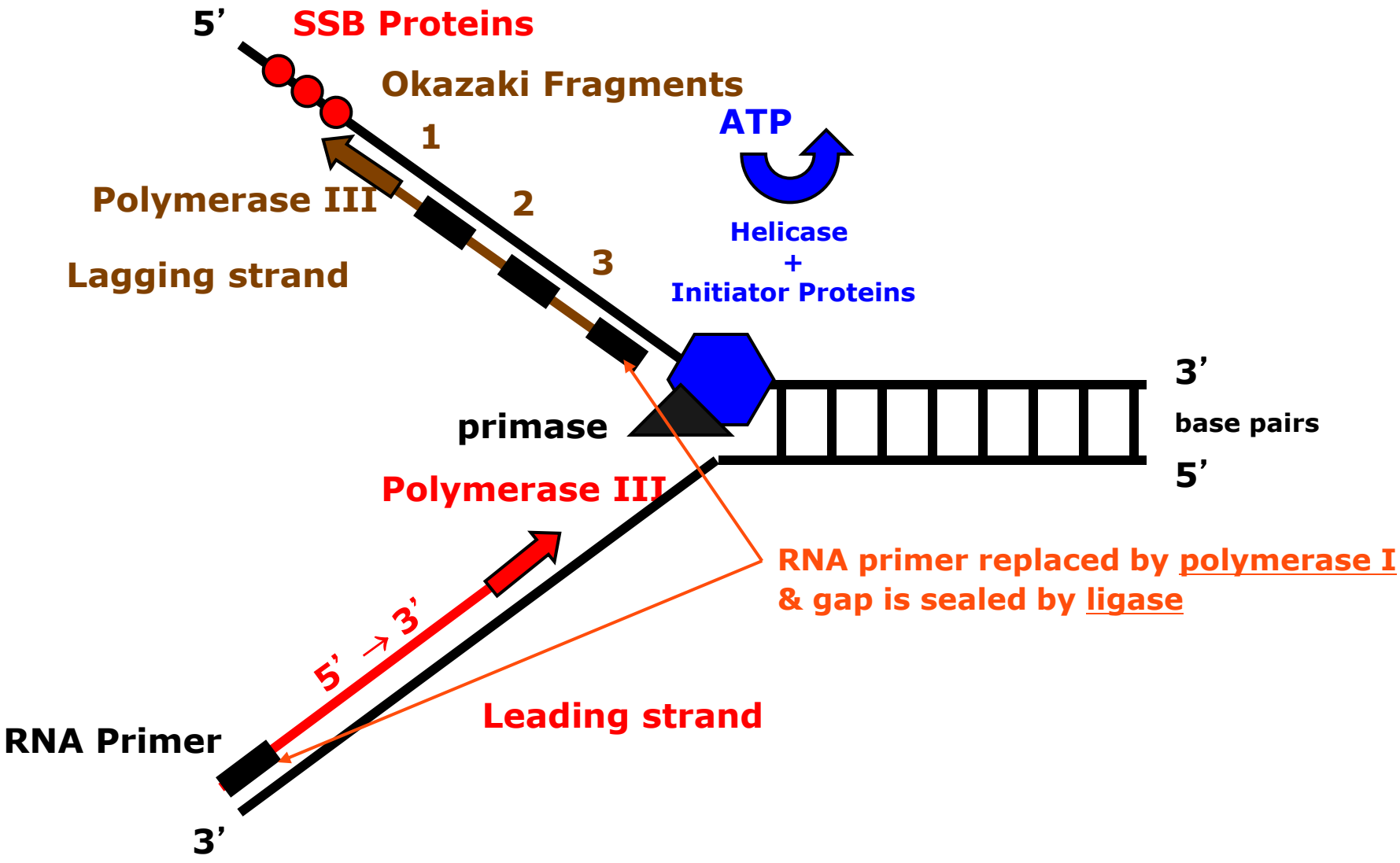
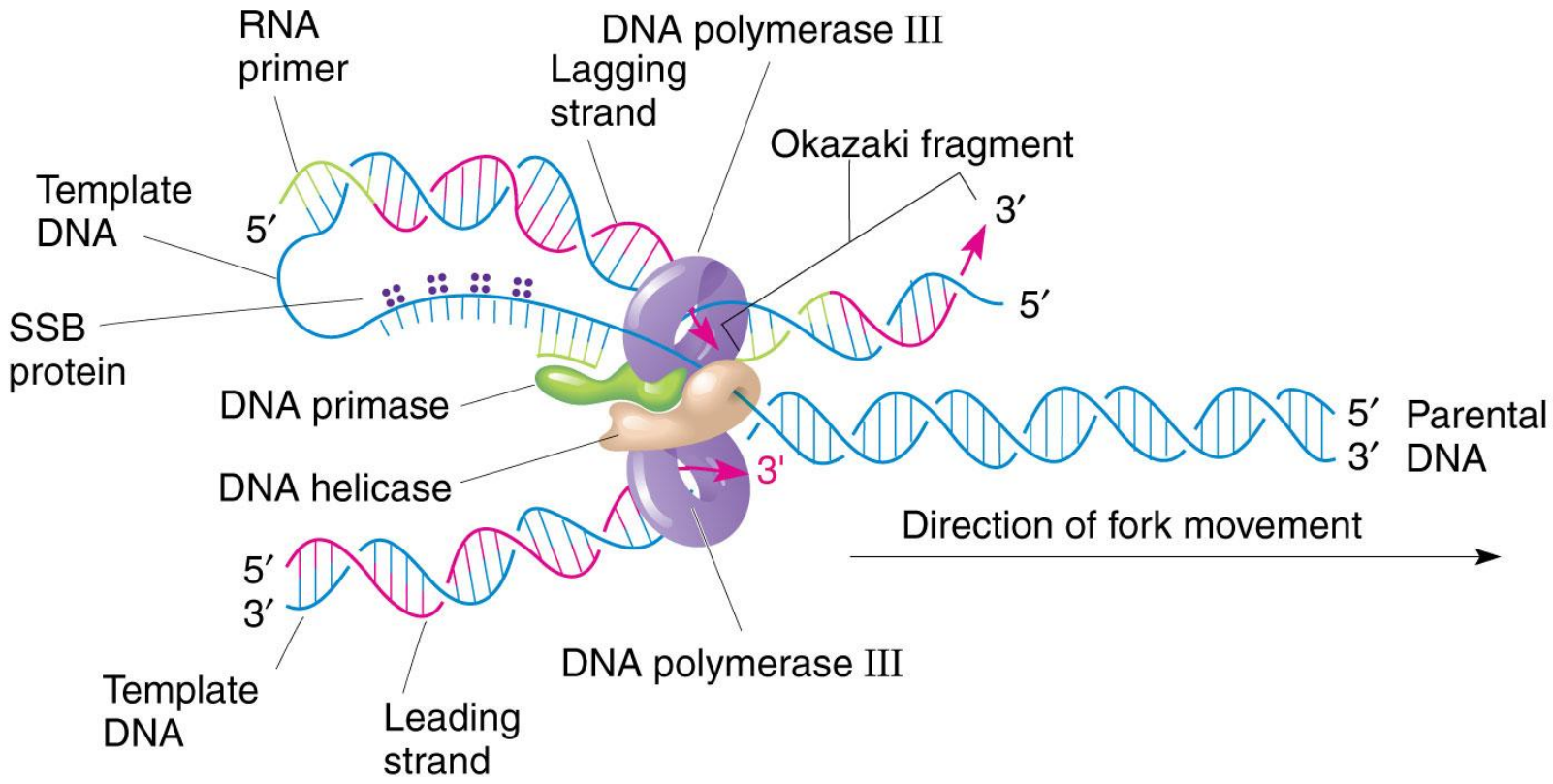


Fig. 3.8 Model of DNA Replication



DNA ligase seals the gaps between Okazaki fragments with a phosphodiester bond (Fig. 3.7)

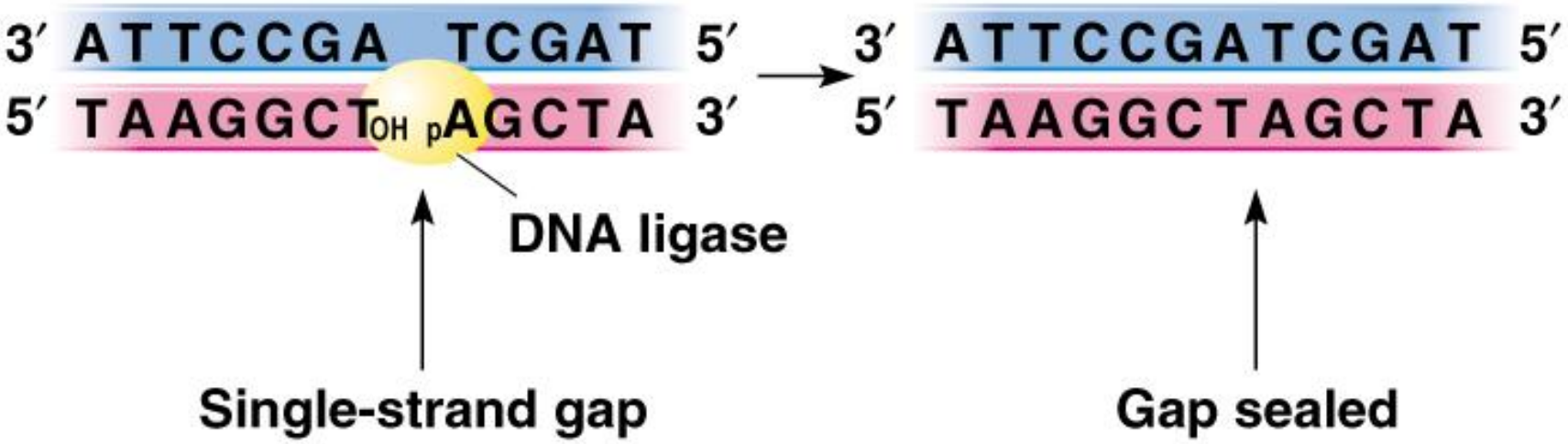


Fig. 3.5 – Model of DNA replication

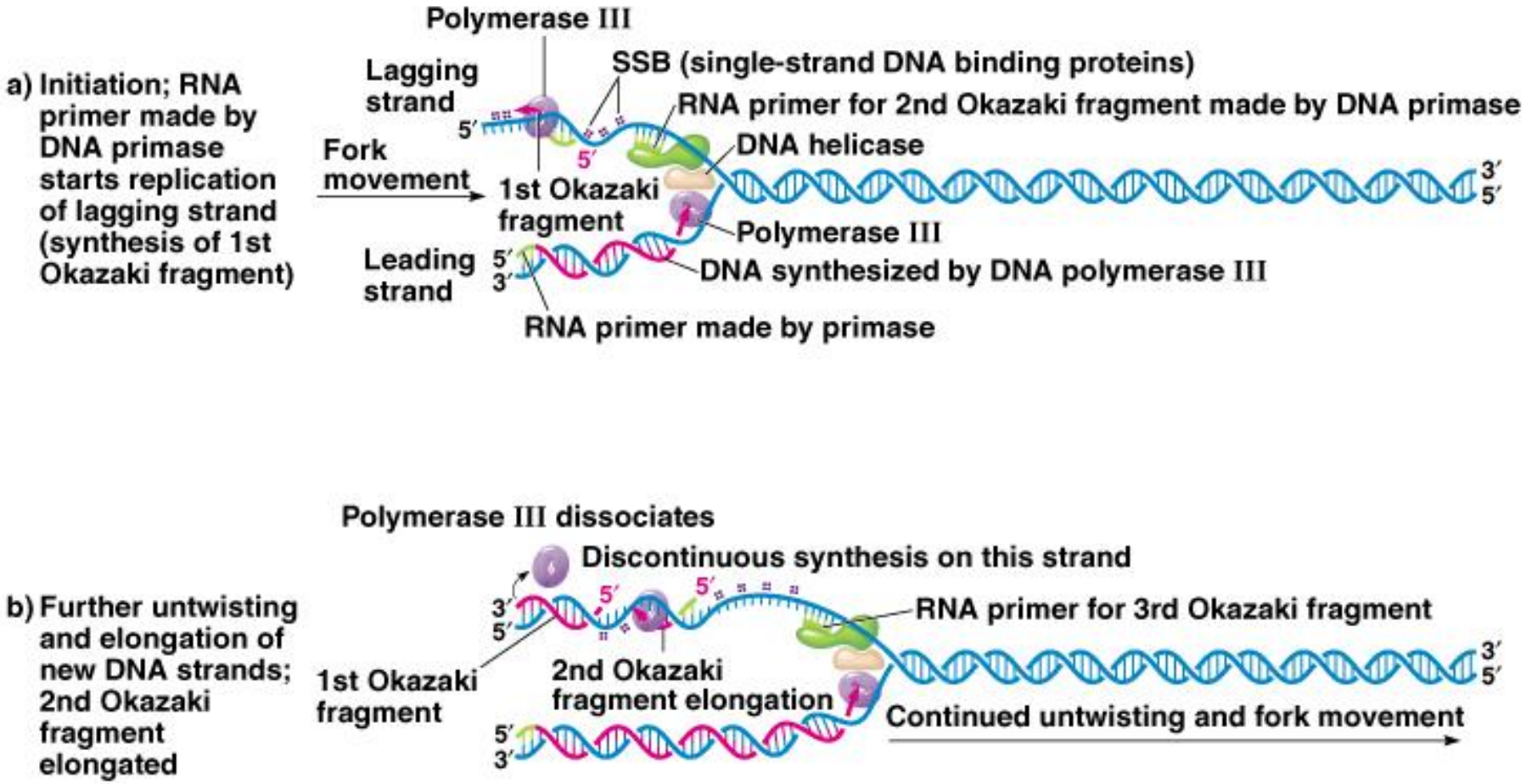
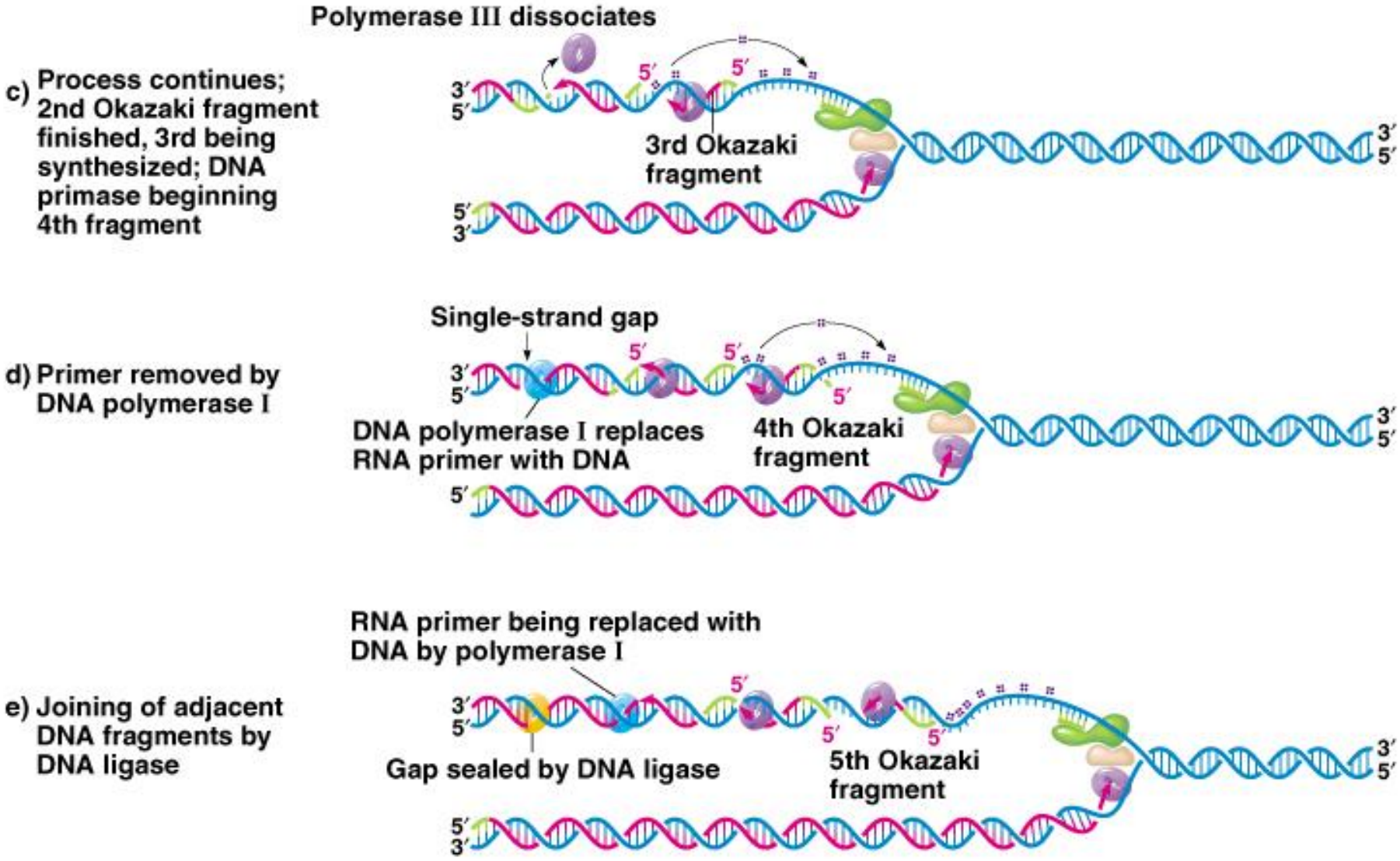


Fig. 3.5 – Model of DNA replication



Concepts and terms to understand:

Why are gyrase and helicase required?

The difference between a template and a primer?

The difference between primase and polymerase?

What is a replication fork and how many are there?

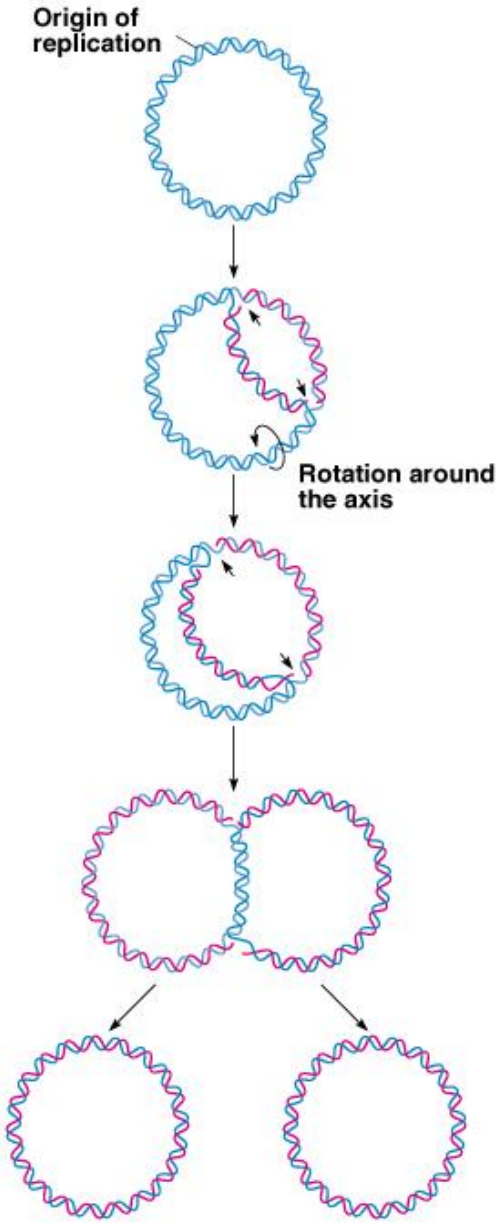
Why are single-stranded binding (SSB) proteins required?

How does synthesis differ on leading strand and lagging strand?

Which is continuous and semi-discontinuous?

What are Okazaki fragments?

How do polymerase I and III differ?

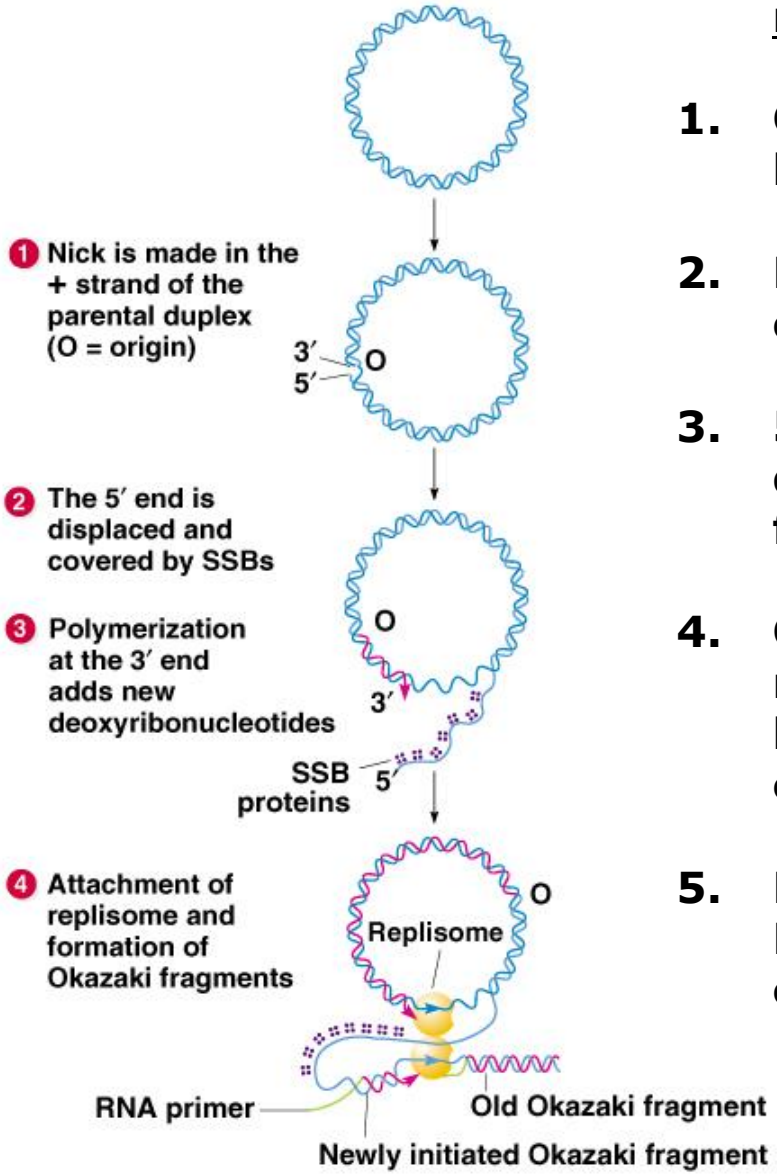


Replication of circular DNA in *E. coli* (3.10):

1. **Two replication forks result in a theta-like (θ) structure.**

2. **As strands separate, positive supercoils form elsewhere in the molecule.**

3. **Topoisomerases relieve tensions in the supercoils, allowing the DNA to continue to separate.**



Rolling circle model of DNA replication (3.11):

1. **Common in several bacteriophages including λ.**
2. **Begins with a nick at the origin of replication.**
3. **5' end of the molecule is displaced and acts as primer for DNA synthesis.**
4. **Can result in a DNA molecule many multiples of the genome length (and make multiple copies quickly).**
5. **During viral assembly the DNA is cut into individual viral chromosomes.**

DNA replication in eukaryotes:

Copying each eukaryotic chromosome during the S phase of the cell cycle presents some challenges:

Major checkpoints in the system

1. **Cells must be large enough, and the environment favorable.**

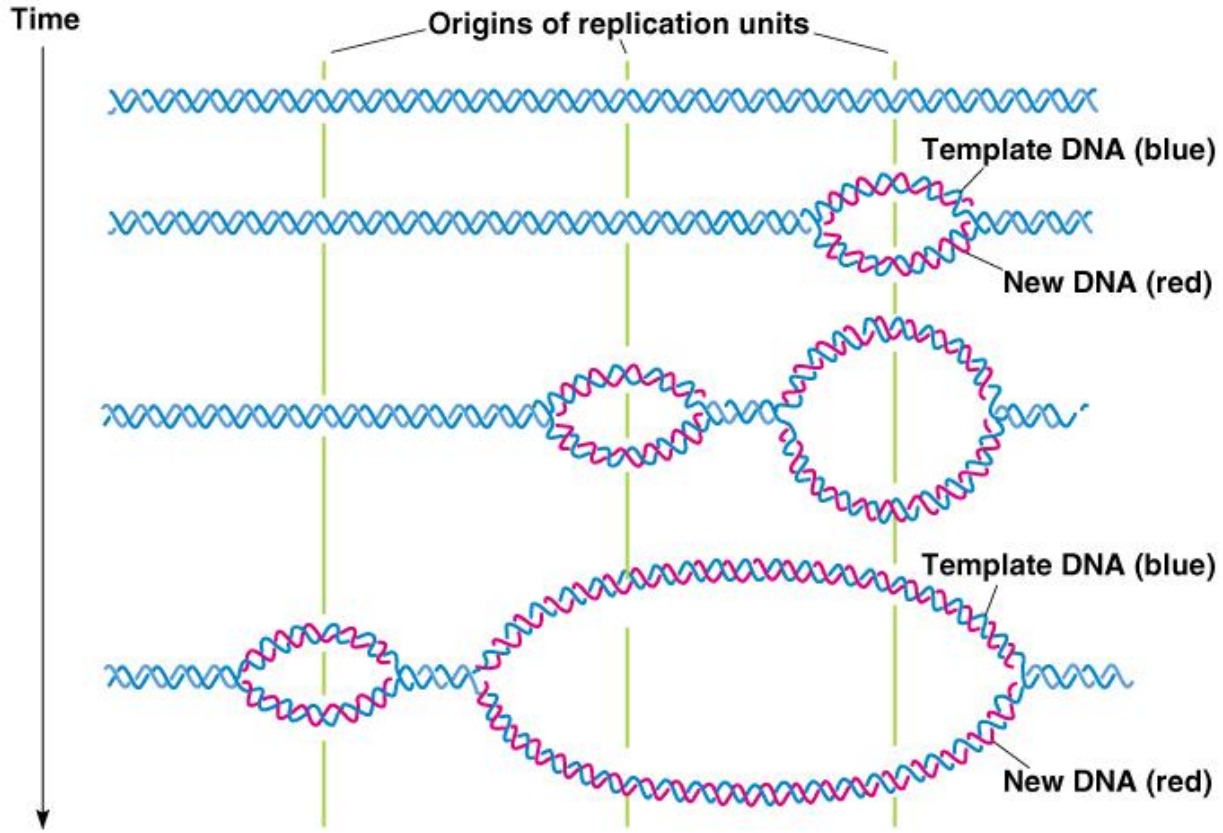
2. **Cell will not enter the mitotic phase unless all the DNA has replicated.**

3. **Chromosomes also must be attached to the mitotic spindle for mitosis to complete.**

4. **Checkpoints in the system include proteins call cyclins and enzymes called cyclin-dependent kinases (Cdks).**

5. **Kinases are enzymes that transfer phosphate groups from donor molecules such as ATP to specific substrates by the process of phophorylation. Important for cell signaling and protein regulation.**

- **Each eukaryotic chromosome is one linear DNA double helix**
- **Average ~10^8 base pairs long**
- **With a replication rate of 2 kb/minute, replicating one human chromosome would require ~35 days.**
- **Solution ---> DNA replication initiates at many different sites simultaneously.**

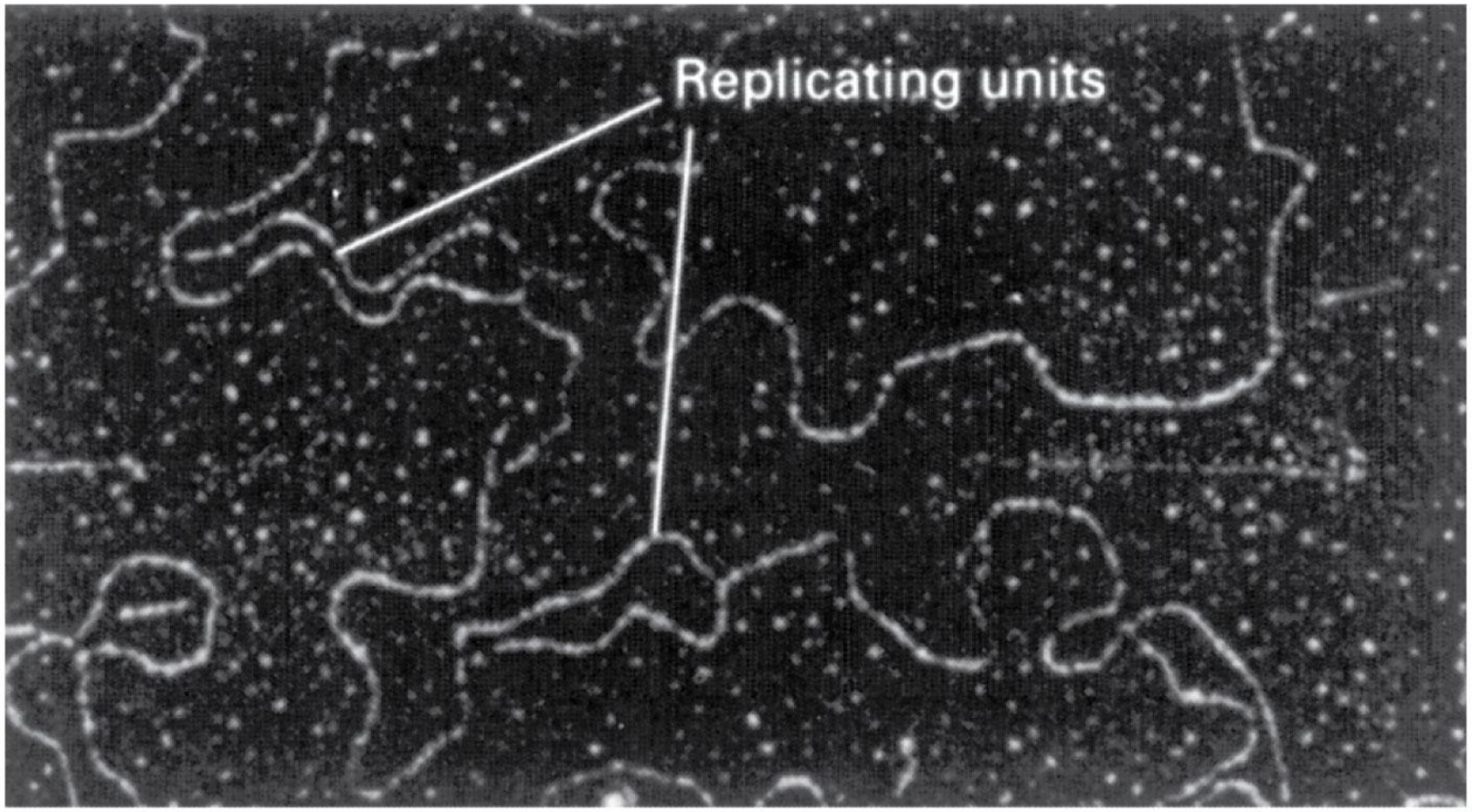


Rates are cell specific!

Fig. 3.14

Fig. 3.13 - Replication forks visible in *Drosophila*

a) Electron micrograph of replicons



What about the ends (or telomeres) of linear chromosomes?

DNA polymerase/ligase cannot fill gap at end of chromosome after RNA primer is removed. If this gap is not filled, chromosomes would become shorter each round of replication!

Solution:

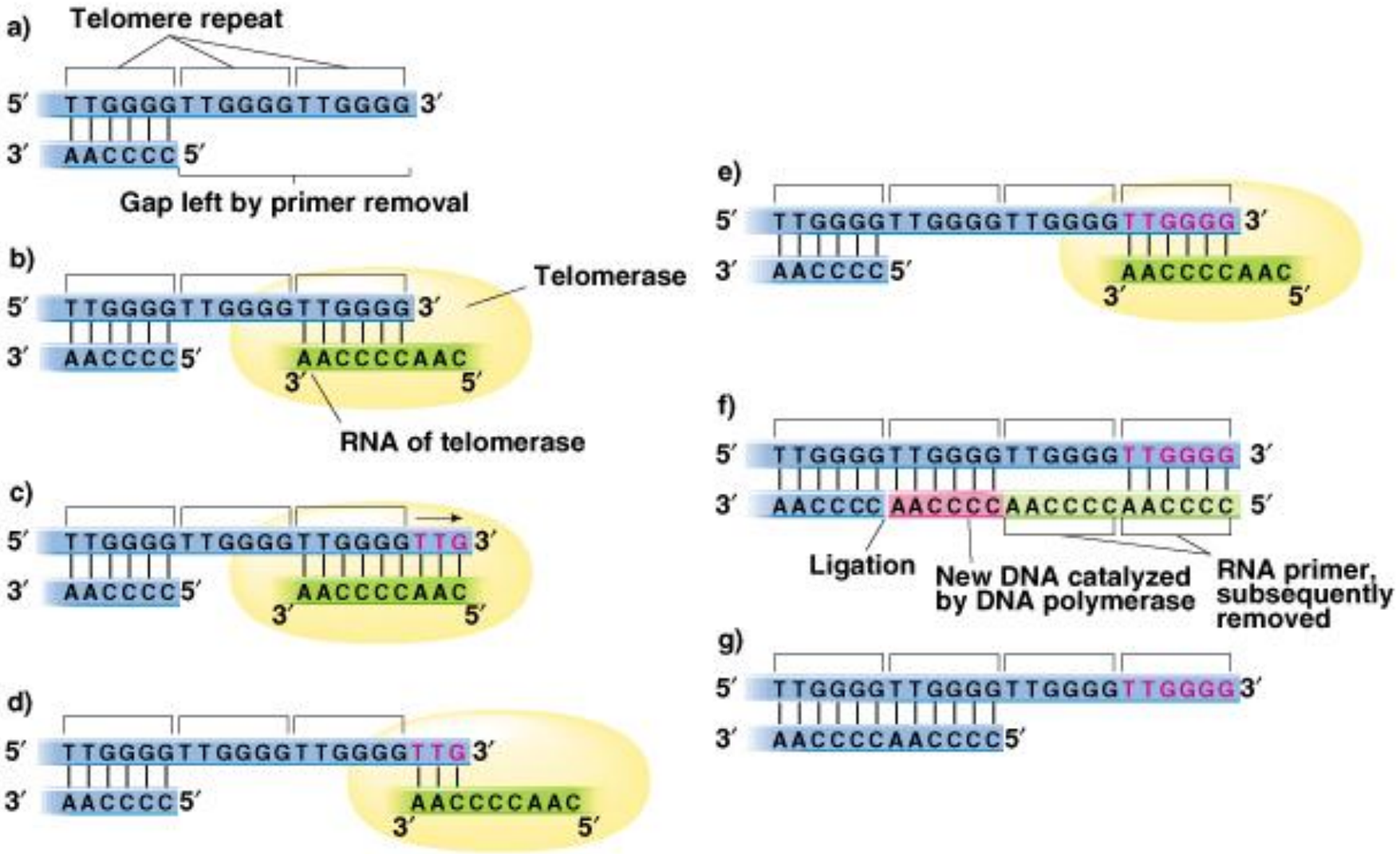
1. **Eukaryotes have tandemly repeated sequences at the ends of their chromosomes.**

2. **Telomerase (composed of protein and RNA complementary to the telomere repeat) binds to the terminal telomere repeat and catalyzes the addition of of new repeats.**

3. **Compensates by lengthening the chromosome.**

4. **Absence or mutation of telomerase activity results in chromosome shortening and limited cell division.**

Fig. 3.16 Synthesis of telomeric DNA by telomerase



Final Step - Assembly into Nucleosomes:

- **As DNA unwinds, nucleosomes must disassemble.**
- **Histones and the associated chromatin proteins must be duplicated by new protein synthesis.**
- **Newly replicated DNA is assembled into nucleosomes almost immediately.**
- **Histone chaperone proteins control the assembly.**

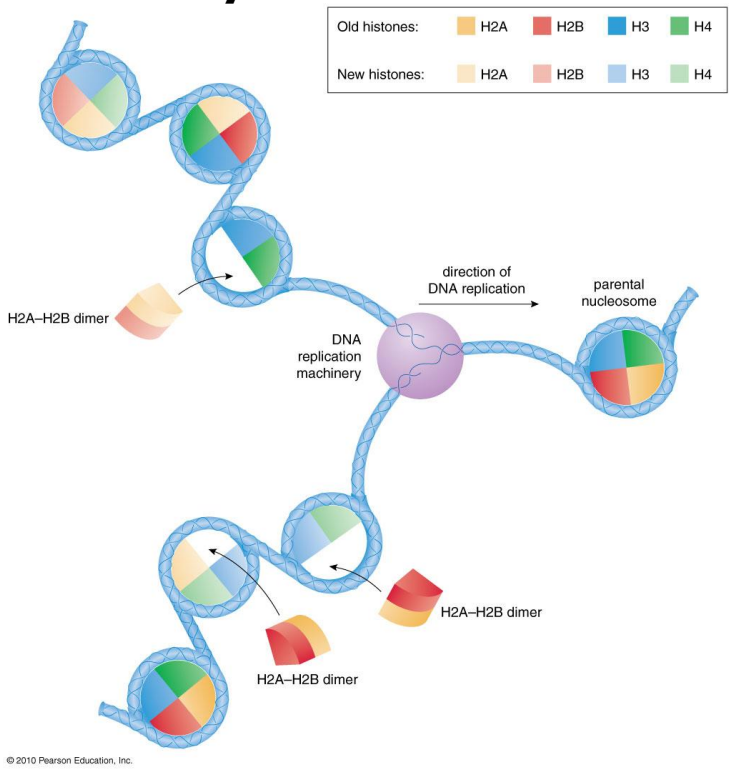


Fig. 3.17

RICK SCOTT,
Florida's GOP Governor

VOLDEMORT,
Harry Potter's Nemesis

SEPARATED AT BIRTH?

Elisabeth Parker


I Hate Trains!
Fuck You I'm Governer!!!