

Topic 8

DNA Replication

Nelson & Cox (2016) Chapter 25, pp. 1009-1027

Nelson & Cox (2017) Chapter 25, pp. 987-1005

You should be able to...

- Describe and use the terms: template, semi-conservative, bi-directional, replication fork, leading strand, lagging strand and proofreading;
- Outline and understand the 'Meselson and Stahl' experiment;
- Describe the replication direction and the differences between the leading and lagging strand synthesis;
- Describe the basic apparatus for replication in prokaryotic cells;
- Understand the difference in replication complexity between eukaryotes and prokaryotes;
- Describe, using diagrams, the different phases of replication

- Structure of nucleic acids, formation of chromosomes (last week)
- DNA replication (this week)
- Transcription (next week)
- Translation (the week after)

- <http://www.youtube.com/watch?v=4PKjF7OumYo>
- <https://www.youtube.com/watch?v=I9ArIJWYZHI>

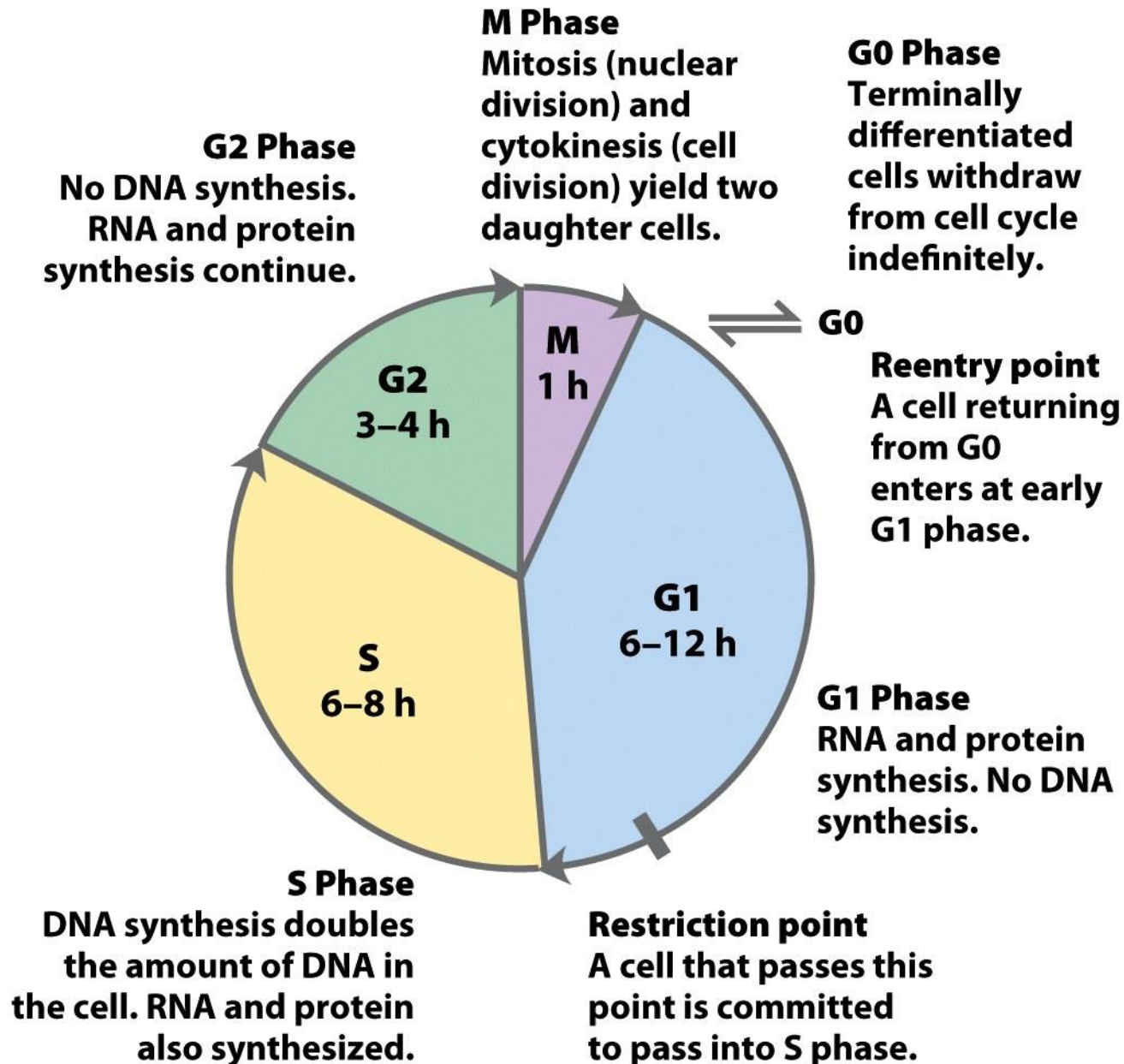
Central Dogma

- DNA gives rise to new DNA via replication
- DNA is copied into RNA via transcription
 - RNA can be copied into DNA via reverse transcription
- RNA is translated into protein via translation

Original theory: DNA → RNA → Protein

Our current understanding: DNA ↔ RNA → Protein

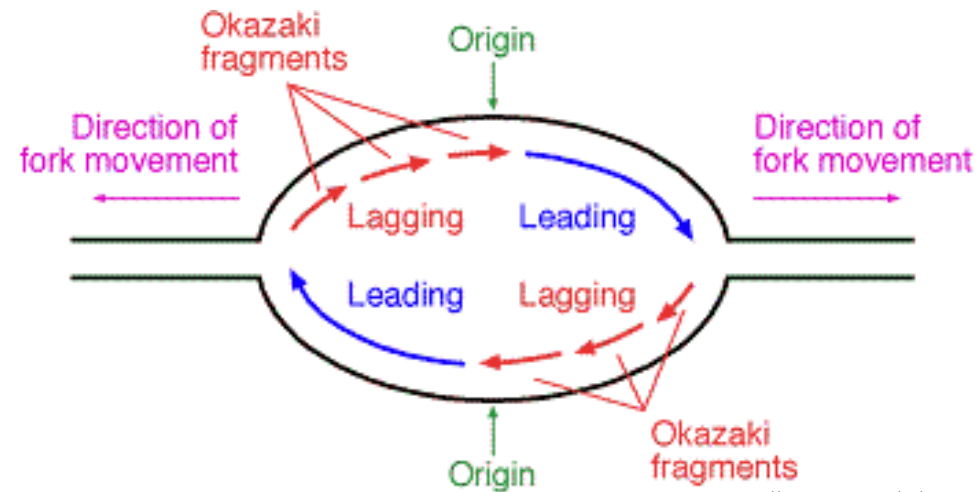
- When is replication needed?
 - The copying of DNA for transfer to progeny cells.
- We know much more about replication in the prokaryotic system of *Escherichia coli* (*E. coli*) than in eukaryotes.



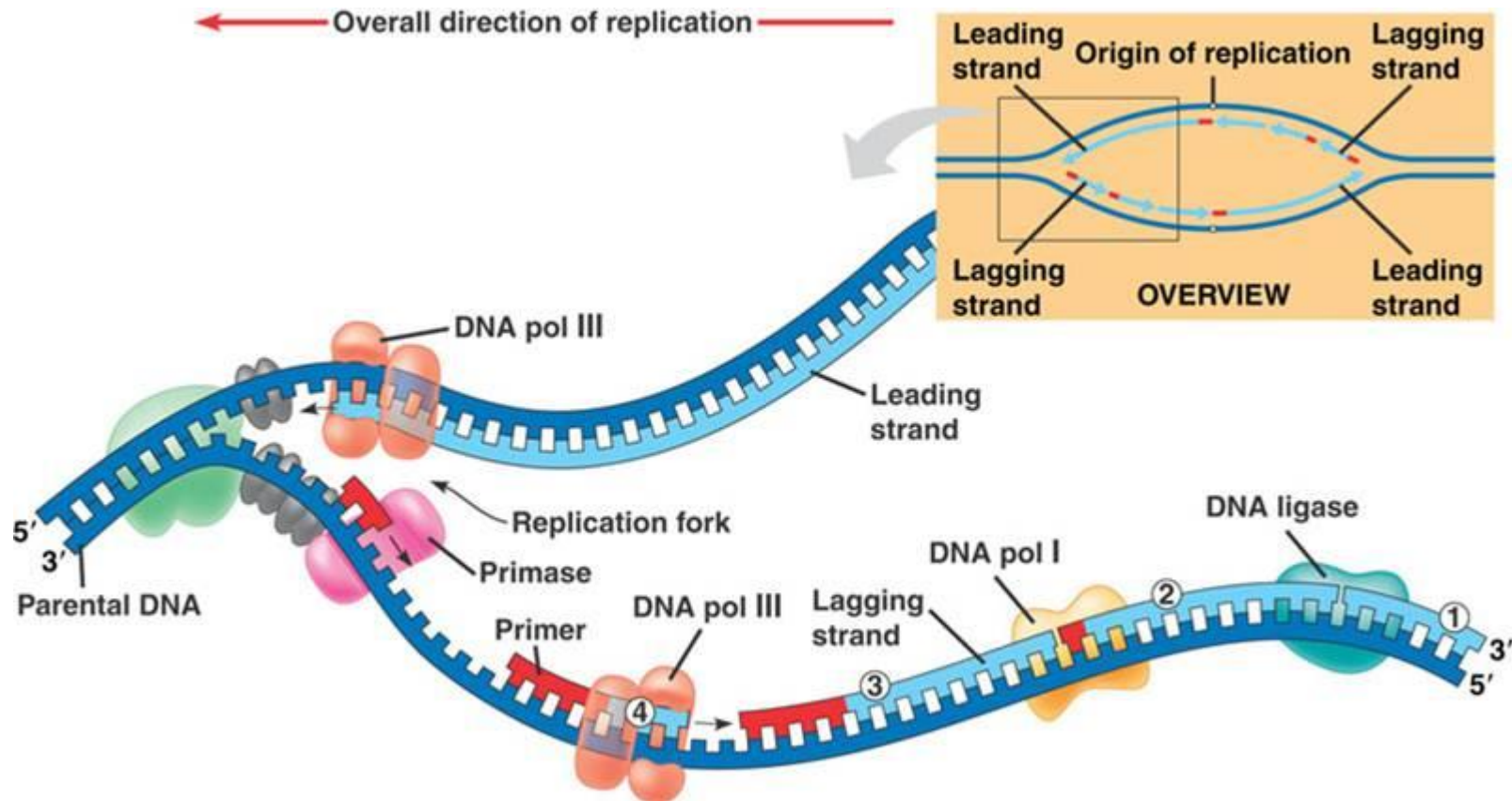
- Summary of features
 - Begins at an **origin of replication**
 - Occurs in **replication forks**
 - Old DNA strand acts as template for the synthesis of a new DNA strand
 - Semi-conservative process
 - Mediated by proteins (enzymes and DNA-binding proteins)

Replication

1. Replication initiated by binding of **DnaA** to ***OriC*** (opens duplex)
2. **Helicase** (DnaB) is loaded by **DnaC** onto the opened duplex at the beginning of each replication fork and begins separating the two helical DNA strands in both directions (**replication bubble**)
3. Binding of **DNA gyrase** prevents supercoiling during unwinding
4. **Single-strand binding proteins (SSB)** bind to ssDNA and protect against digestion, premature annealing and the formation of secondary structures
5. RNA primer inserted by **primase** to allow binding of **DNA polymerase**
6. **DNA polymerase (III)** synthesises new DNA strands in a **5'→3'** direction that are **complementary** to the template strand
7. **Leading strand** is synthesised continuously
8. **Lagging strand** is synthesised discontinuously (**Okazaki fragments**)
9. RNA primers are removed by **RNase H** (or equivalent) and replaced with DNA by **DNA polymerase (I)**
10. Okazaki fragments joined by **DNA ligase**
11. Replication finishes at the **termination sequence (Ter)**



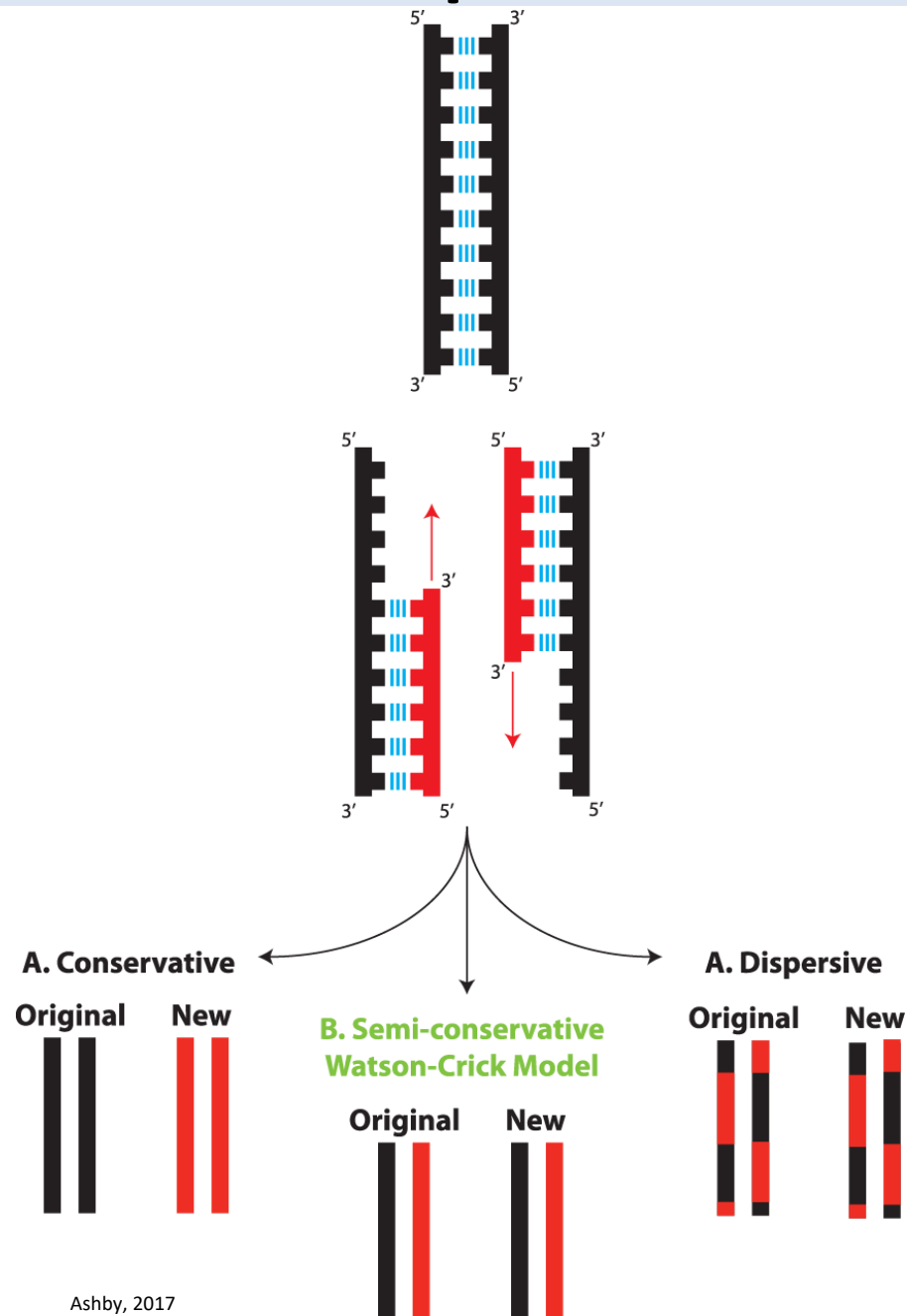
Replication



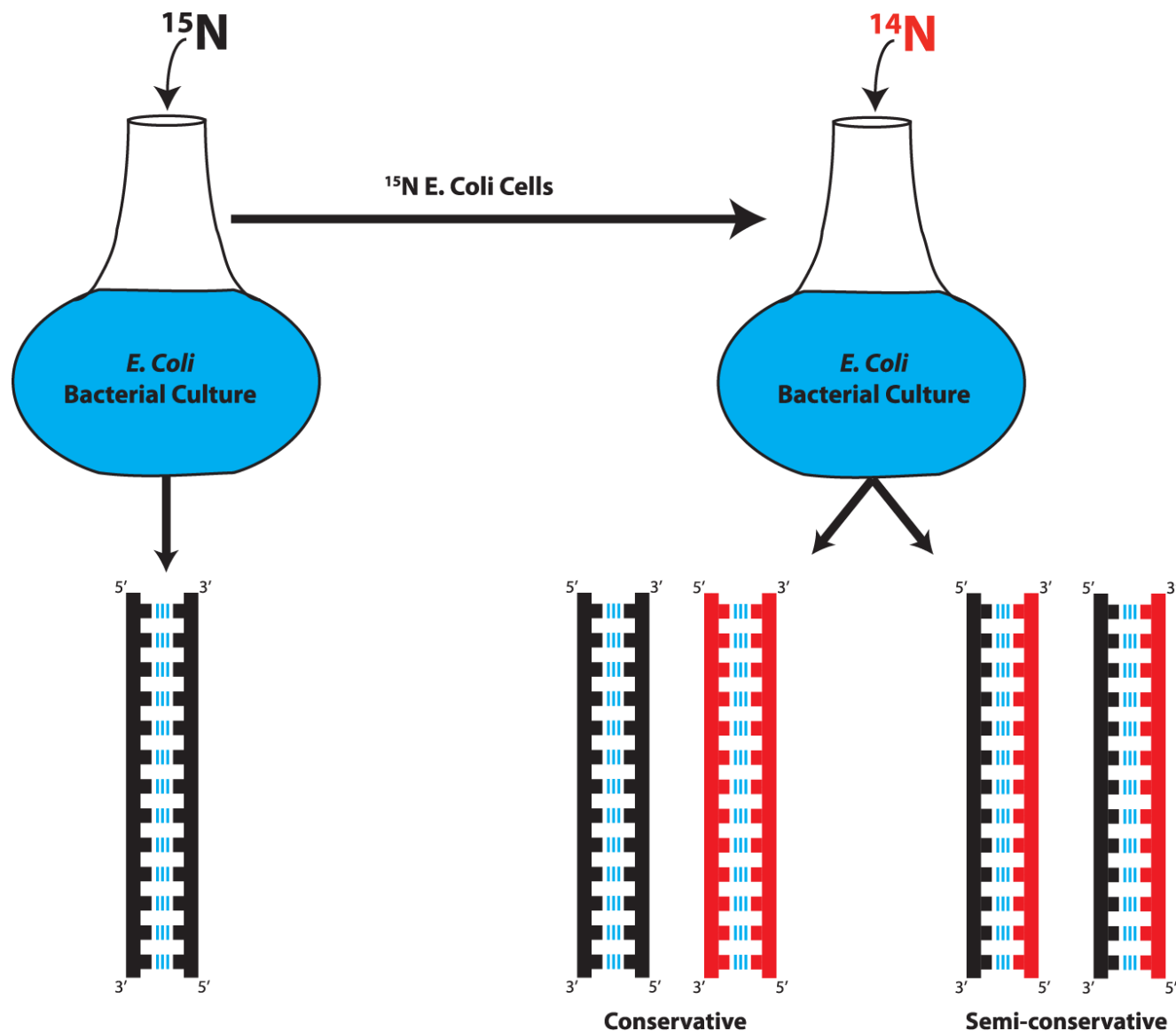
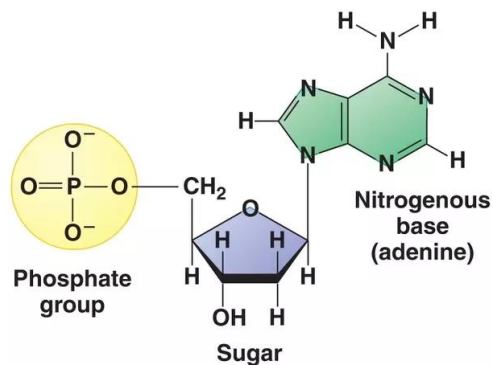
<http://genmed.yolasite.com/fundamentals-of-genetics.php>

NOTE: Such figures are made to give students a good overview, but are not a true representation of the process

DNA Replication



Meselson-Stahl Experiment

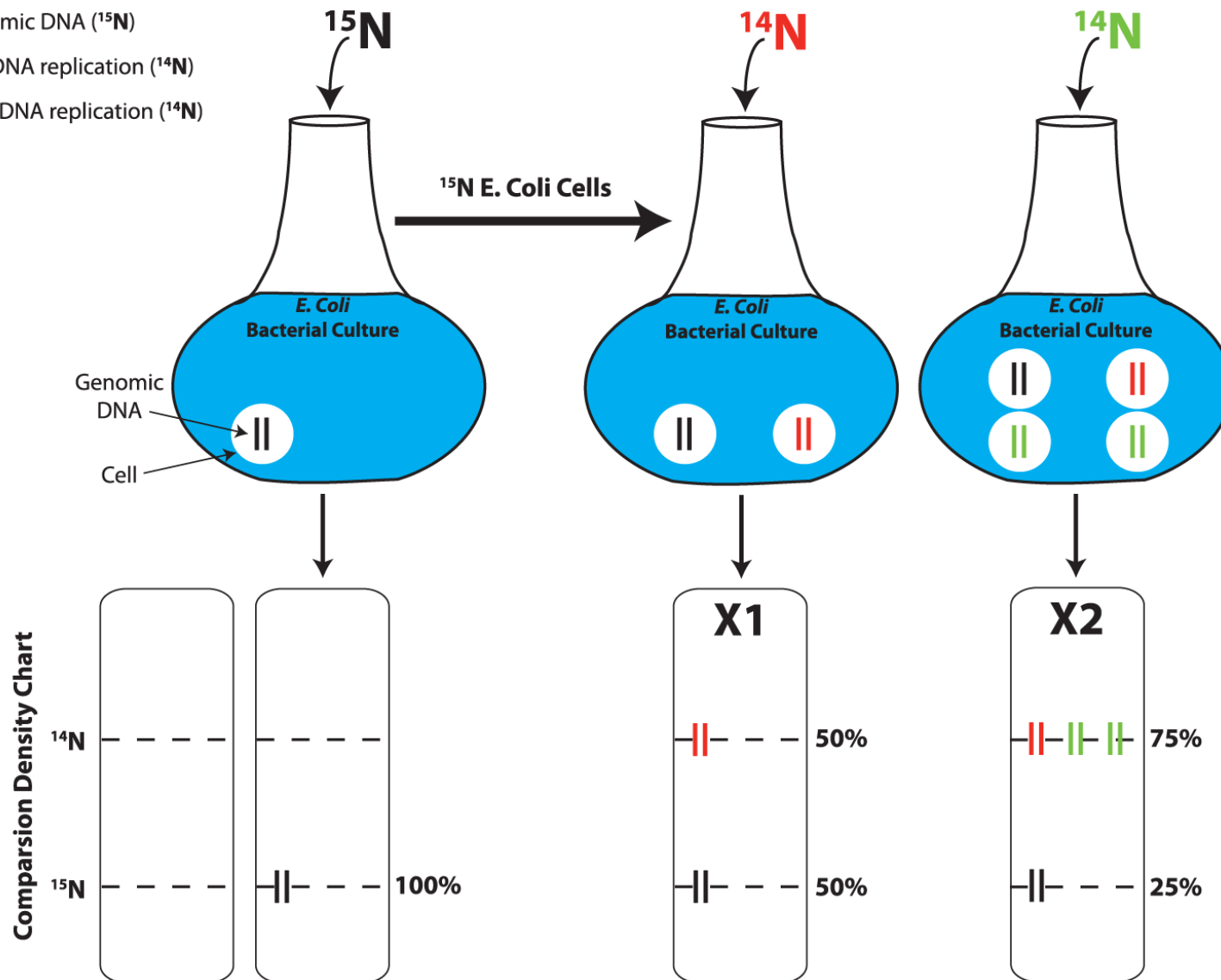


Conservative Replication

| = Original genomic DNA (^{15}N)

| = 1st round of DNA replication (^{14}N)

| = 2nd round of DNA replication (^{14}N)

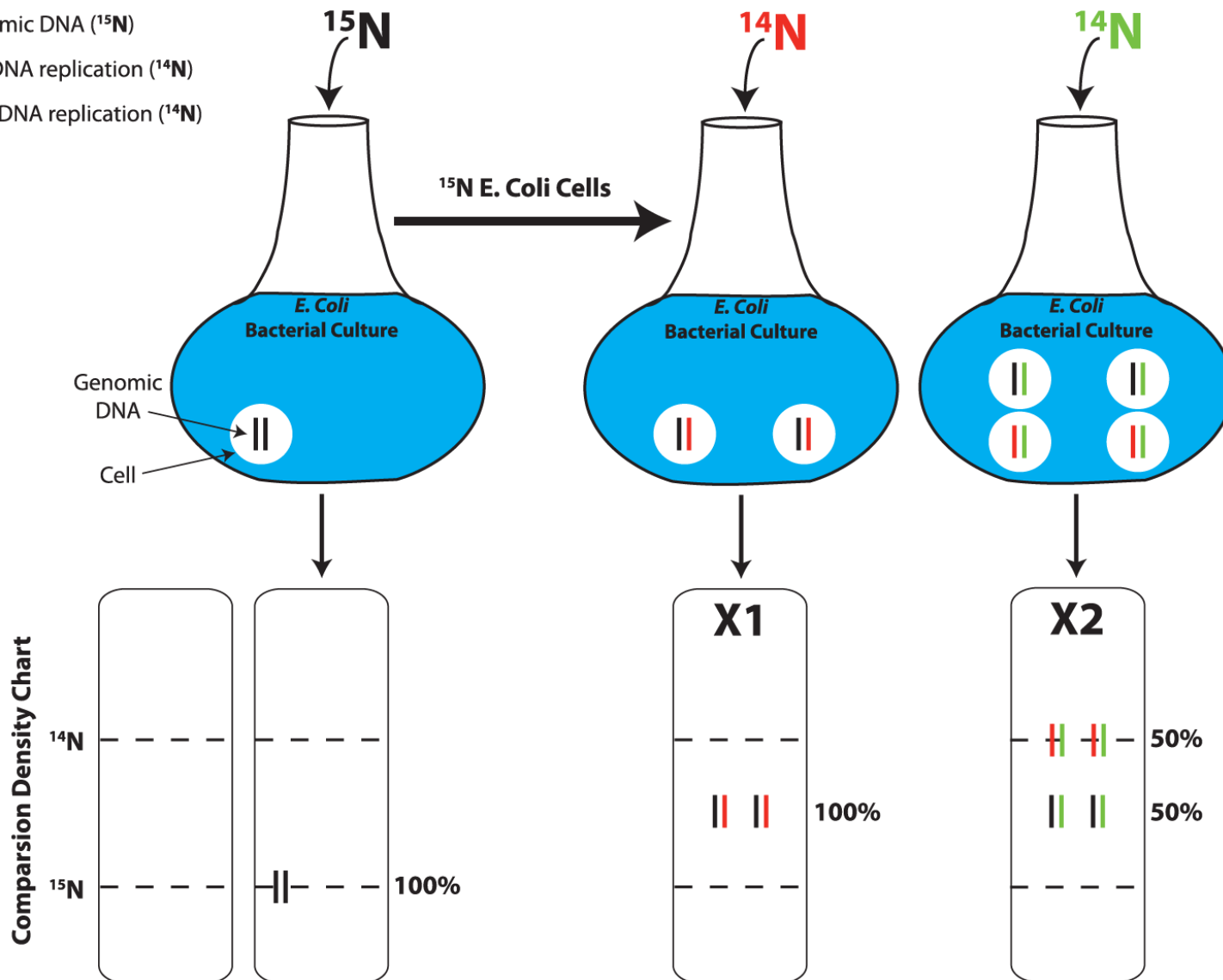


Semi-conservative Replication

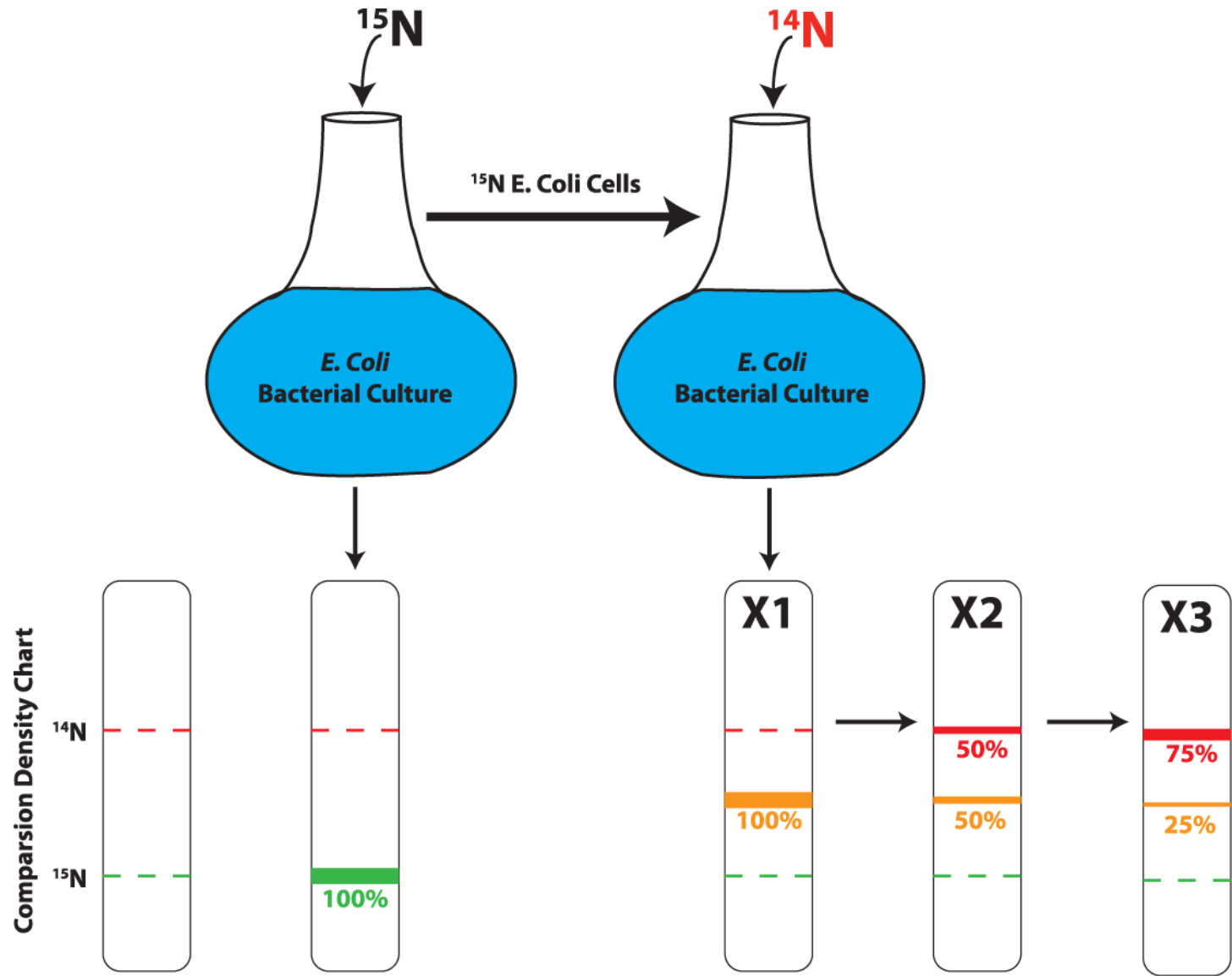
| = Original genomic DNA (^{15}N)

| = 1st round of DNA replication (^{14}N)

| = 2nd round of DNA replication (^{14}N)



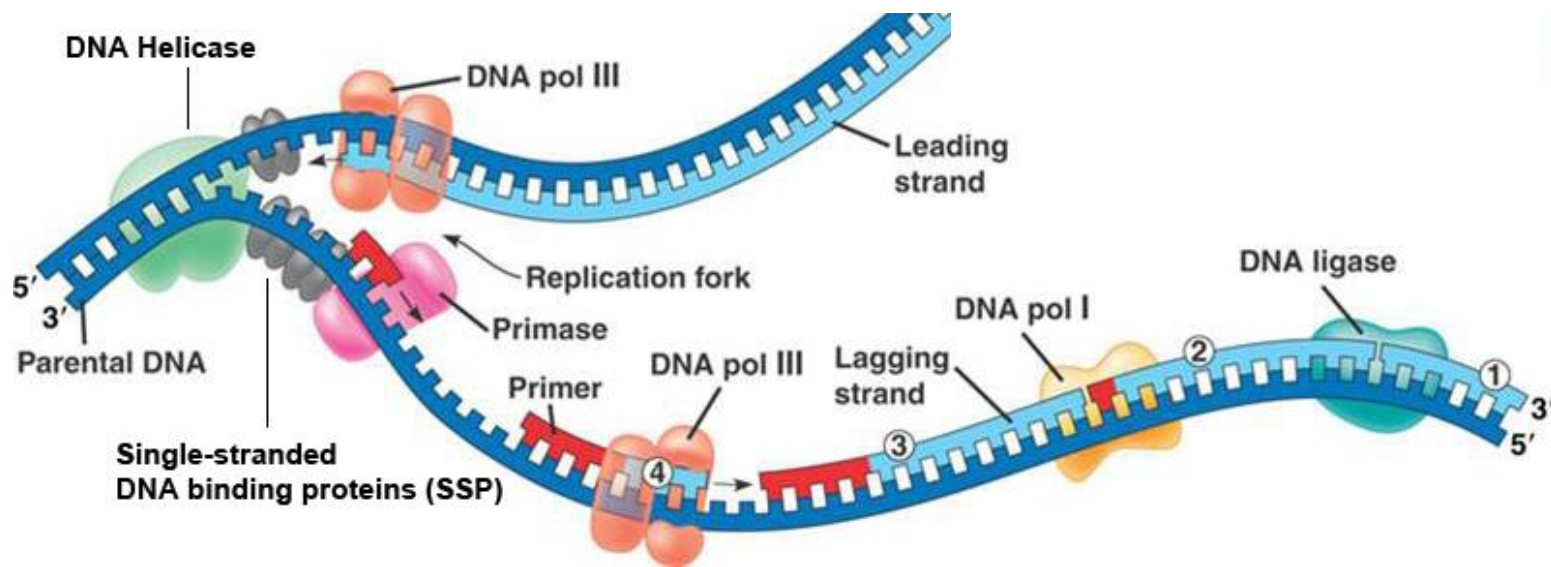
Meselson-Stahl Experiment



Ashby, 2012

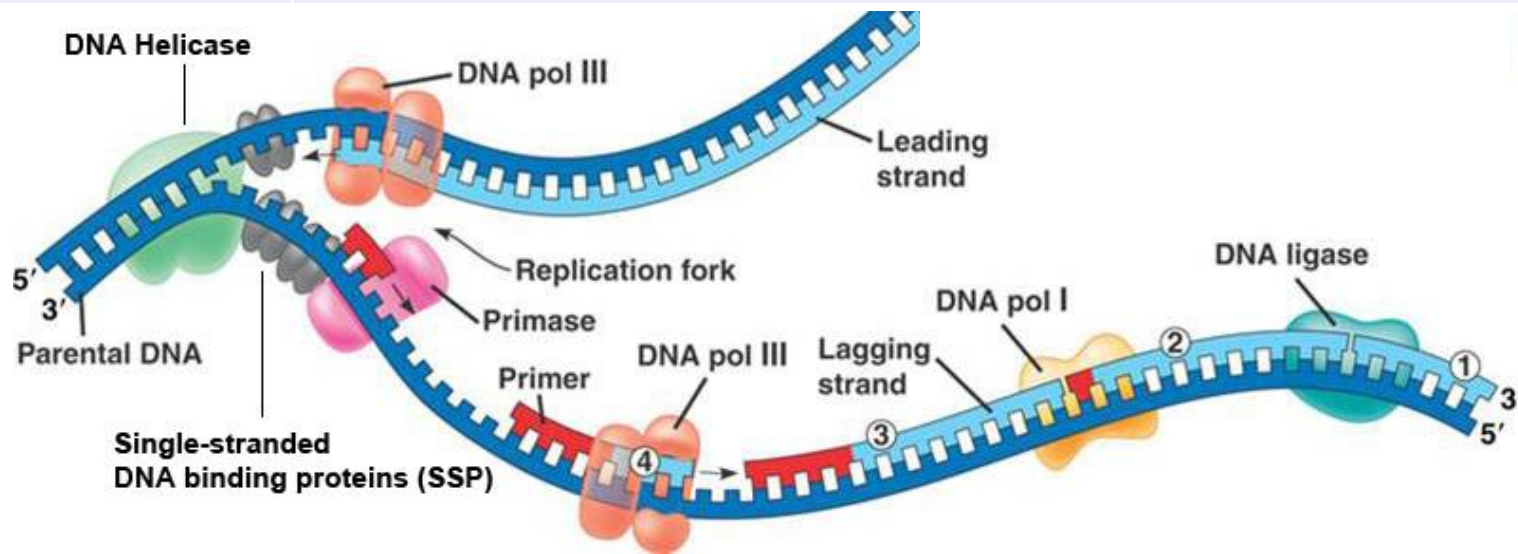
Proteins Required for Replication

Protein	Function
DnaA	Binds to origin of replication (<i>OriC</i>), initiates replication in <i>E. coli</i>
DnaB (Helicase)	Unwinds DNA helical structure
DnaC	Required for DnaB binding at origin
Single Stranded Binding Proteins (SSB)	Binds single stranded DNA (ssDNA), preventing re-annealing, degradation and formation of secondary structures
DnaG (Primase)	Synthesises short RNA primers to initiate replication. DNA polymerase can only add nucleotides to a pre-existing 3' OH group

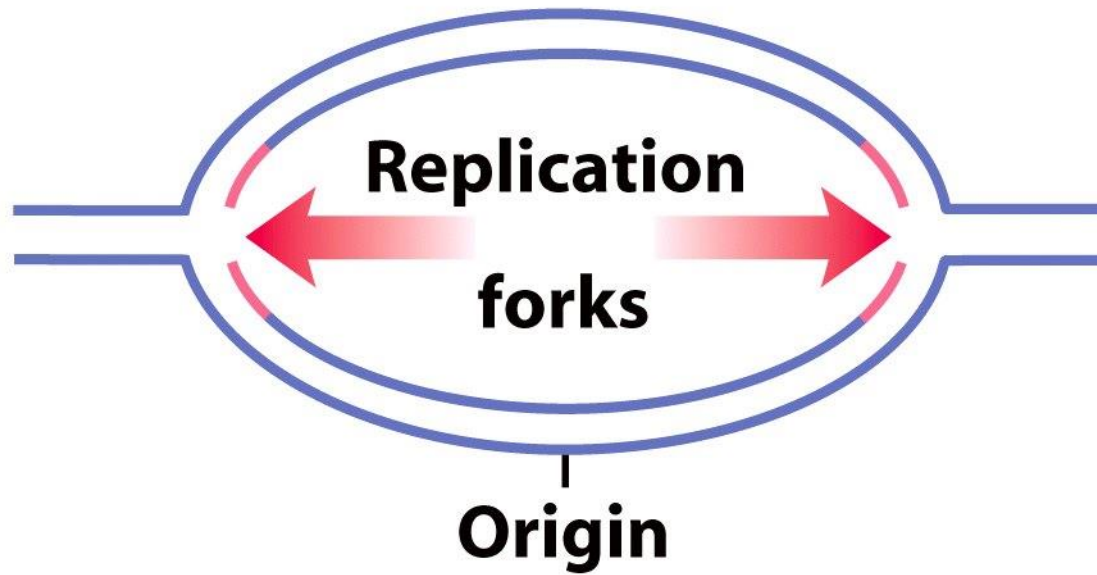


Proteins Required for Replication

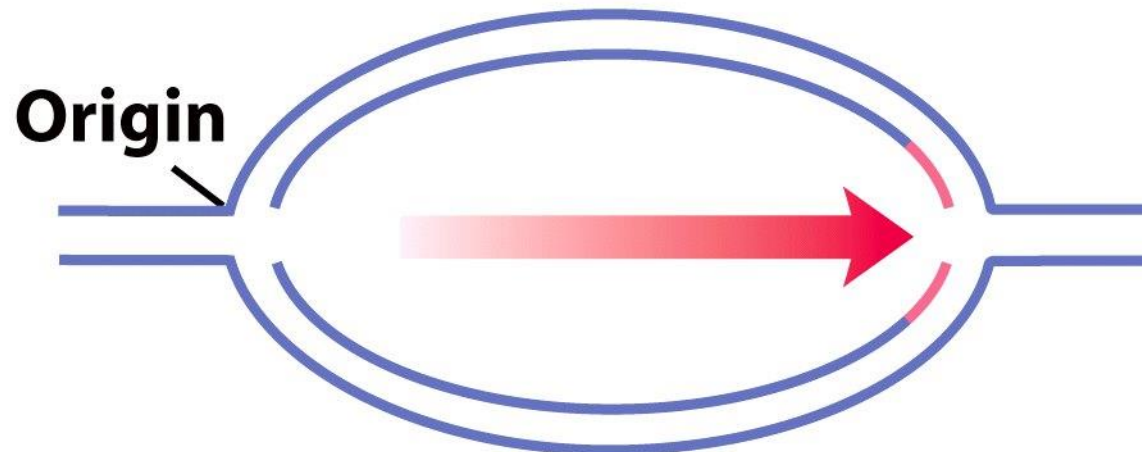
Protein	Function
DNA Gyrase (Topoisomerase II)	Reduces torsional strain (supercoiling) that occurs during DNA unwinding
DNA Polymerase III	Catalyses elongation of the growing (new) DNA strand through the addition of nucleotides (dNTP) to the free 3' OH end (5'→3')
RNase H (or equivalent)	RNA endonuclease that removes (cleaves) RNA primers
DNA Polymerase I	Replacement of RNA primers with DNA, proofreading abilities
DNA Ligase	Catalyses the joining (ligation) of two DNA segments
Initiation Factors (HU, FIS, IHF)	Stimulate initiation of replication

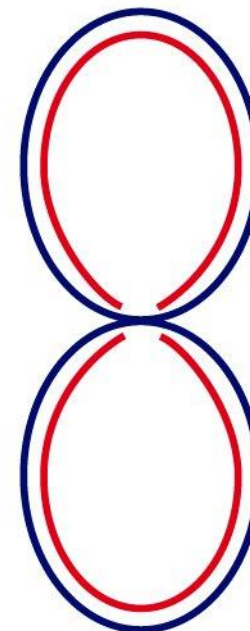
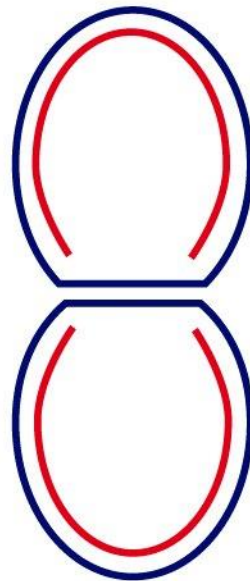
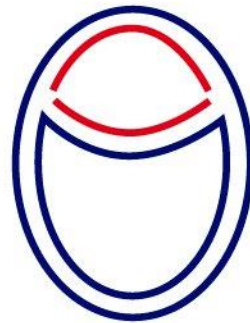
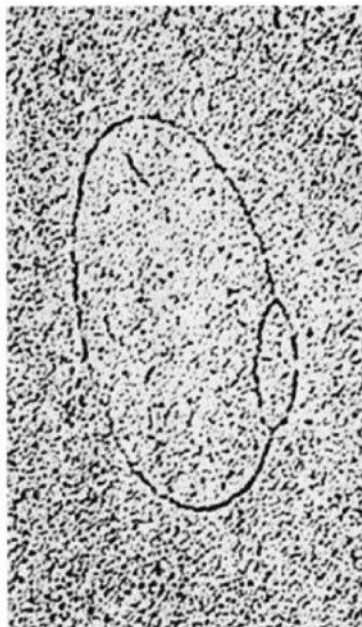


Bidirectional *



Unidirectional





Three Phases of Replication

1. Initiation

- The beginning
- Regulated

2. Elongation

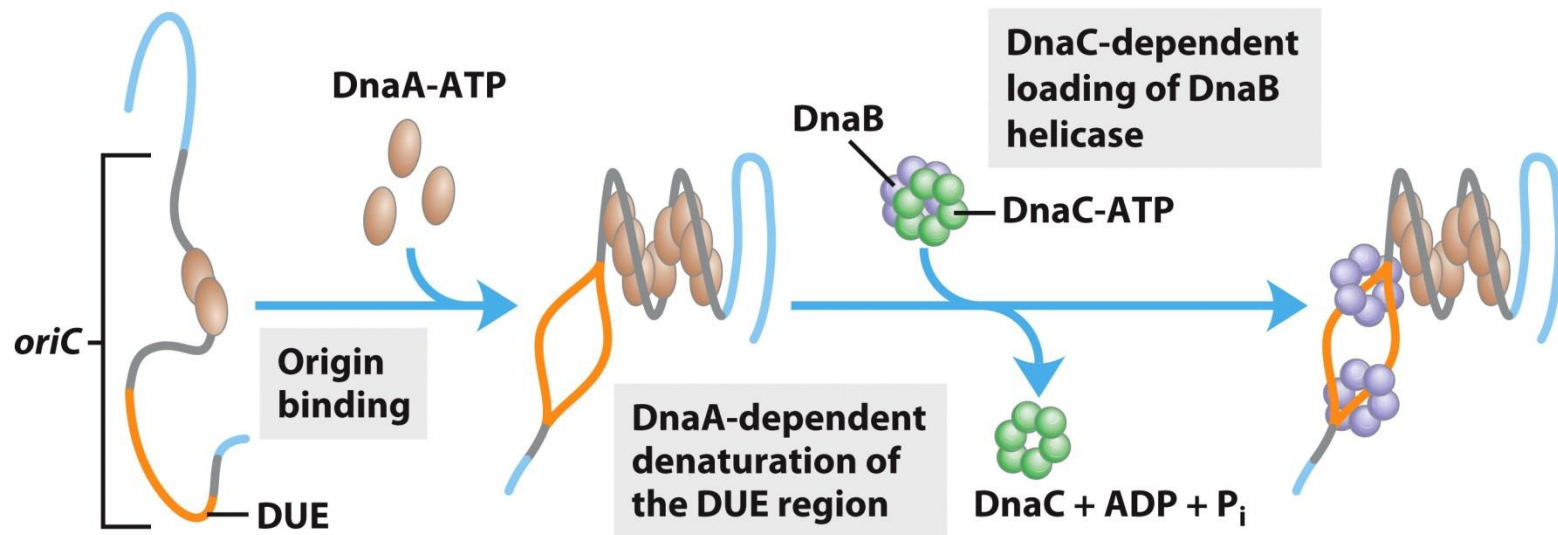
- Synthesis of new strands

3. Termination

- The end

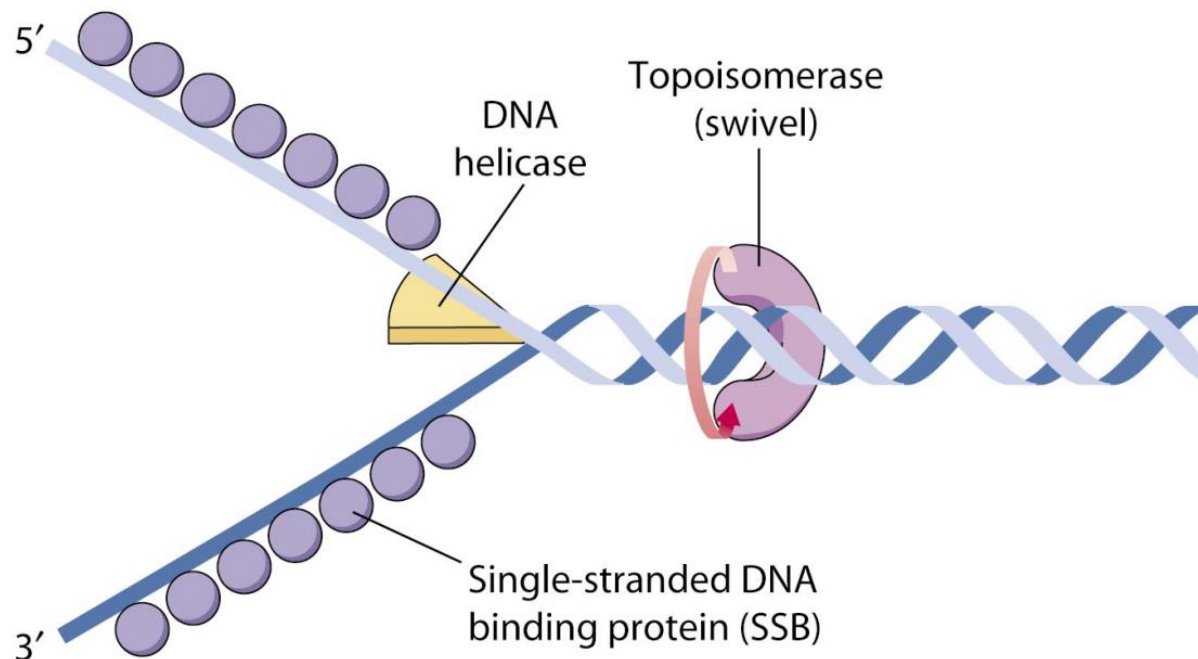
Initiation

- **Origin (*E. coli*)**
 - Origin of replication (***OriC***), 245 bp region of DNA
 - Contains binding sites for **DnaA**
- Eight DnaA molecules bind at *OriC*, assembling to form a right-handed helical structure, with the aid of initiation factors
- The helical strain causes denaturation of an A=T rich region, referred to as the **DNA unwinding element (DUE)**, leading to opening of the helix and separation of the strands

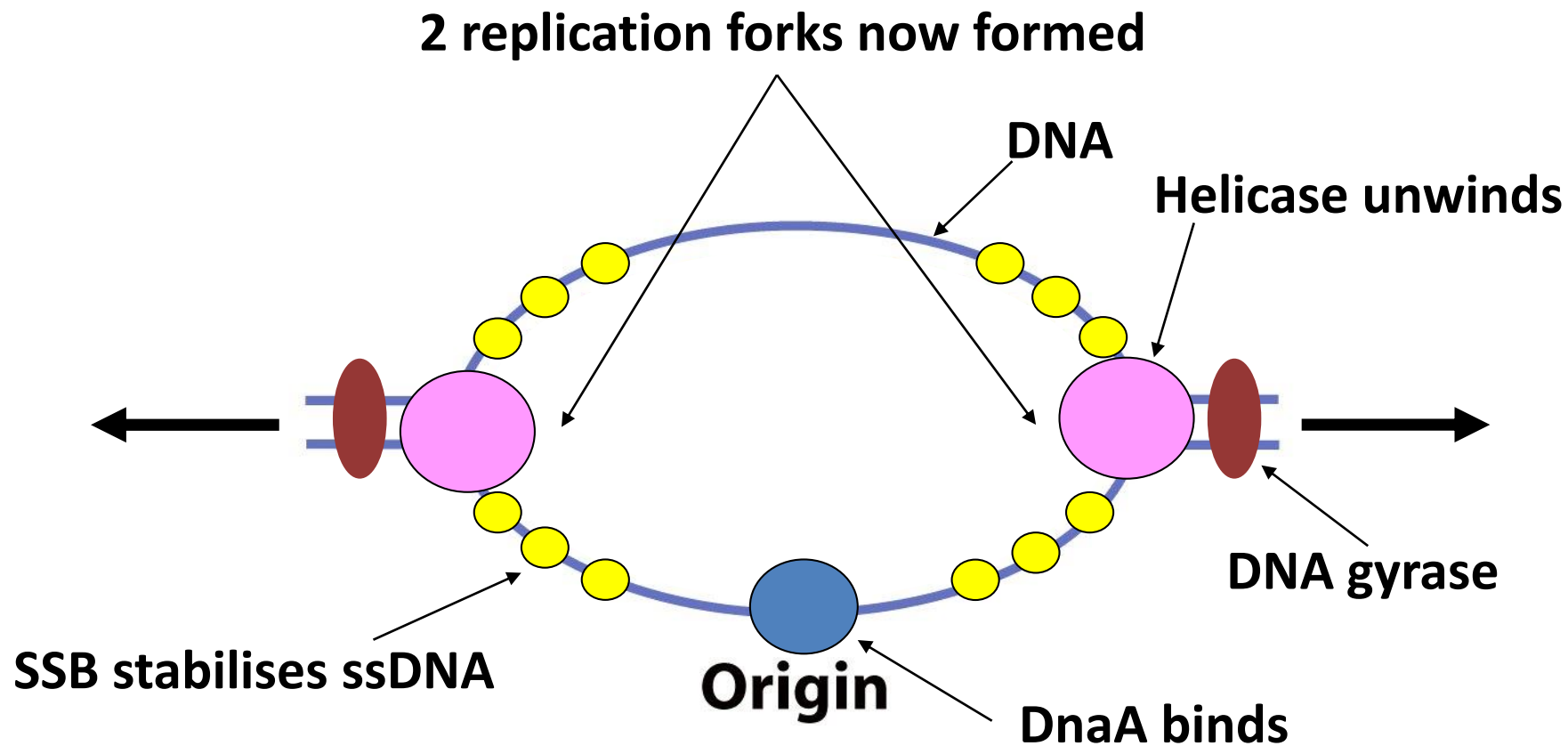


Initiation

- **Helicase (DnaB)** is loaded (binds) onto the separated DNA strands with the aid of **DnaC**. DnaB unwinds the strands and produces 2 replication forks (bi-directional).
- DNA gyrase (topoisomerase II) relieves torsional strain (supercoiling)
- **SSB's** bind to **ssDNA**, produced through unwinding, stabilising the ssDNA

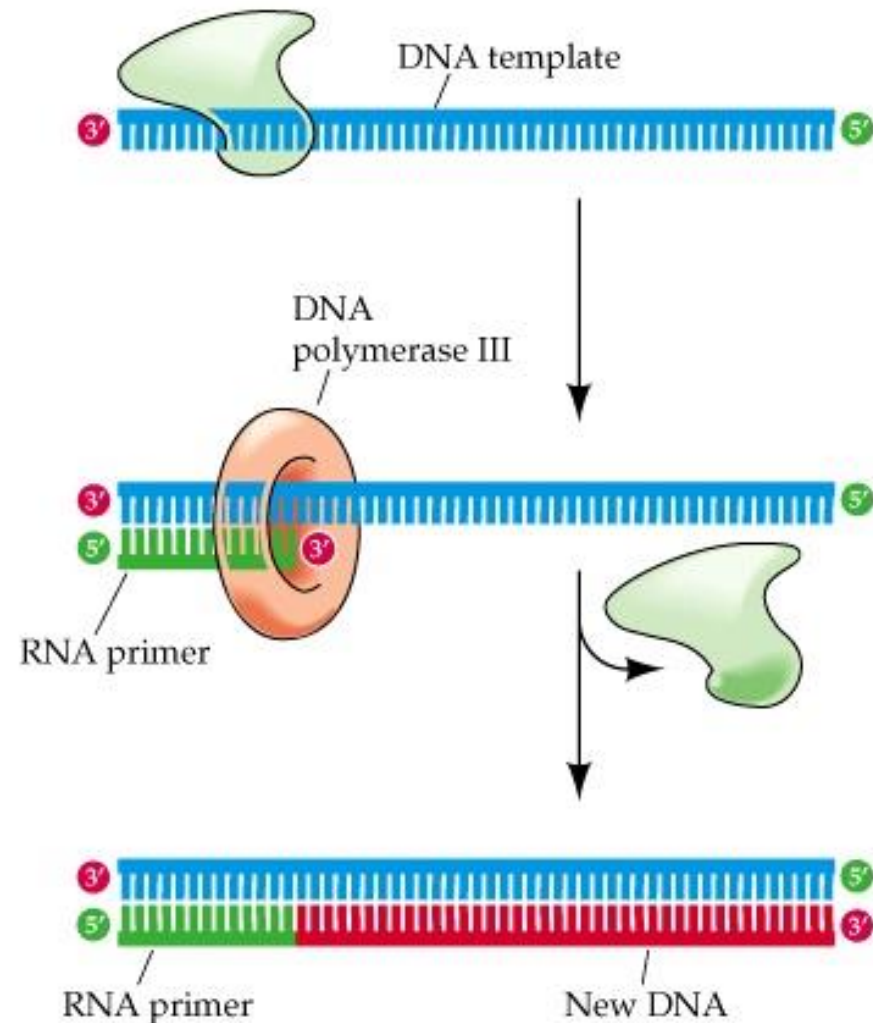


Initiation



Elongation

- Following DNA strand separation:
 - **Primase** creates small RNA primer to initiate replication (10-12 nucleotides long)
 - **NOTE:** DNA polymerase can only add nucleotides to a pre-existing free 3' OH-end
 - **DNA polymerase (III)** binds the RNA primer and elongates a new reverse complementary DNA strand either in a continuous (leading strand) or non-continuous (lagging strand) manner in a 5' → 3' direction.



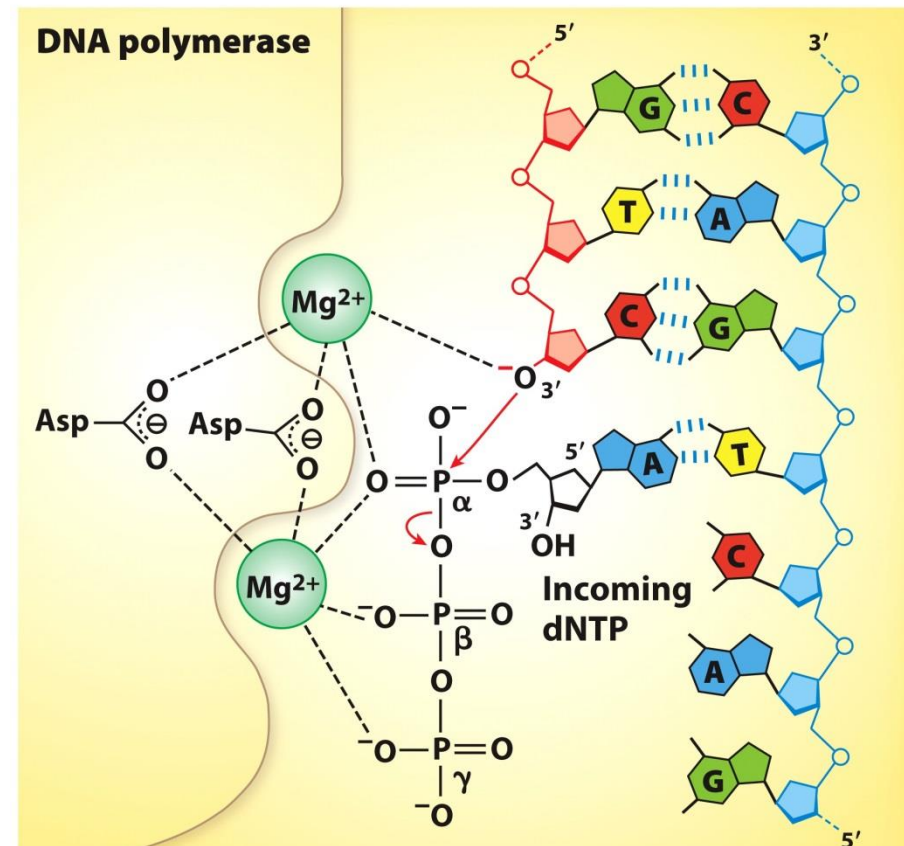
Elongation

- **Leading strand (continuous elongation):** DNA polymerase (III) binds RNA primer and elongates a new DNA strand continuously in a $5' \rightarrow 3'$ direction as the DNA is unwound ahead.
- **Lagging strand (non-continuous elongation):** moving towards the replication fork, the new DNA strand would be synthesised in a $3' \rightarrow 5'$ direction.
 - DNA pol. Can only synthesis in a $5' \rightarrow 3'$
 - Therefore, to allow DNA pol. to travel in the $5' \rightarrow 3'$ direction, the new DNA strand is made in 'instalments' (**Okazaki fragments**).
 - Prokaryotes (1,000-2,000 nt long), eukaryotes (100-200 nt long)
 - **RNase H** (or equivalent) and **DNA polymerase (I)** remove and replace RNA primers with DNA.
 - **DNA Ligase** joins Okazaki fragments together

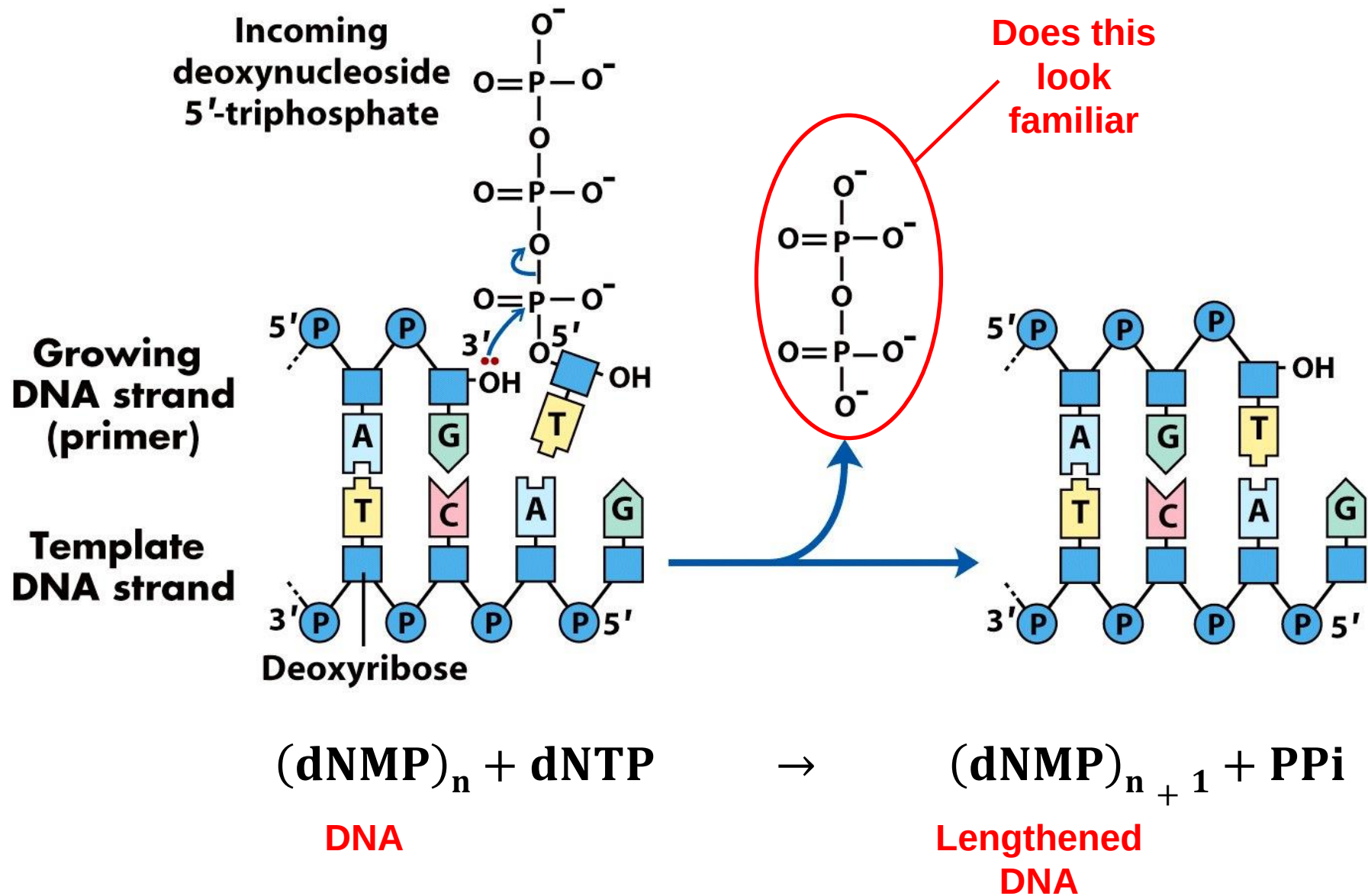
Elongation

- DNA polymerase extends the new strand through the addition of nucleotides, from deoxynucleotide triphosphates (dNTPs), to the growing strand
 - dATP, dCTP, dGTP, dTTP (building blocks and energy source)
 - Using the complimentary base-pairing rules, DNA pol. catalyses the formation of a phosphodiester bond between the free 3' hydroxyl (OH) group on the growing DNA strand and the dNTP α -phosphate
 - DNA pol. requires Mg^{2+} ions as cofactors. The first helps facilitate the 3' –OH group nucleophilic attack on the α -phosphate. The second helps stabilise and displace the pyrophosphate (di-phosphate).

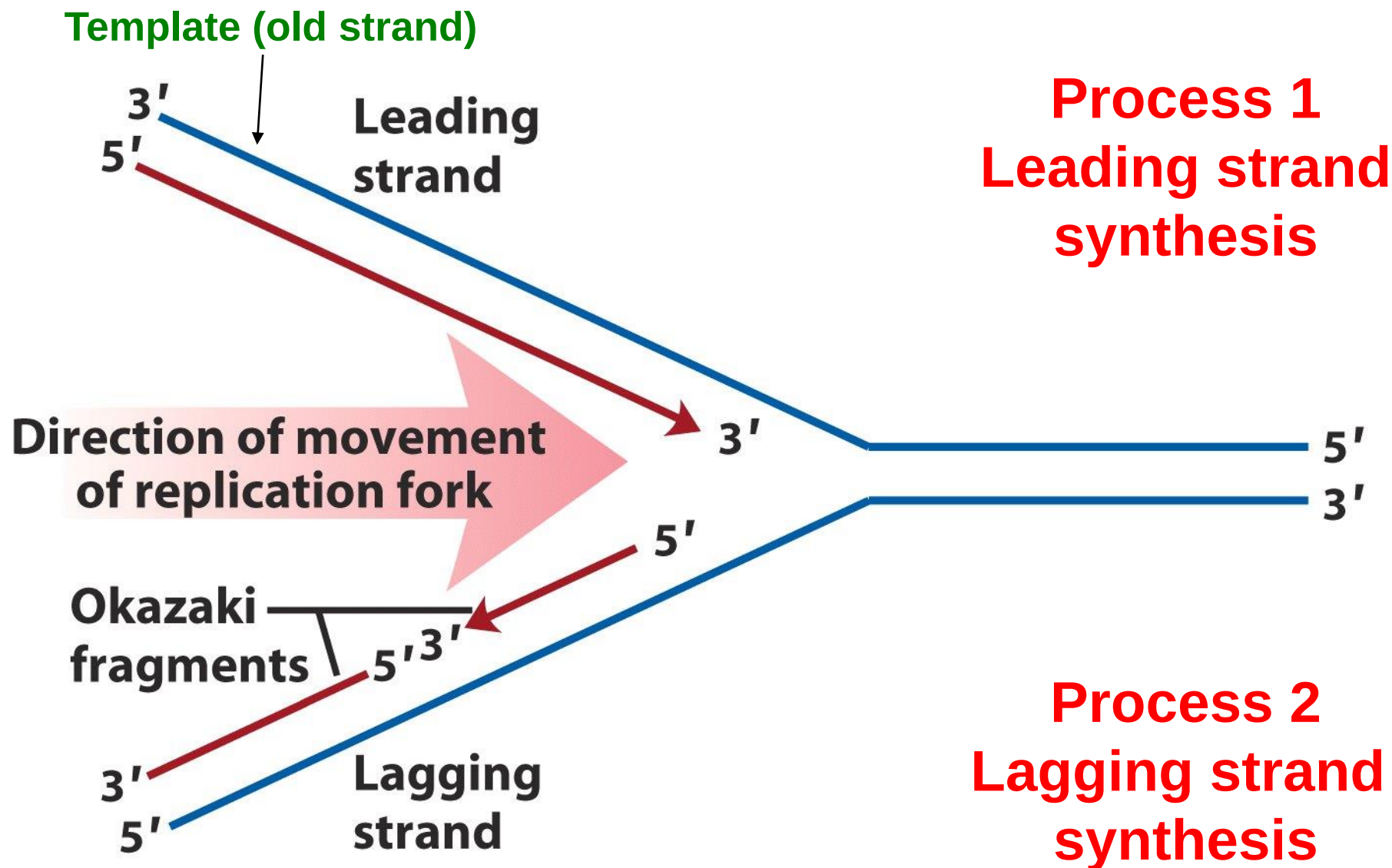
DNA synthesis



Elongation

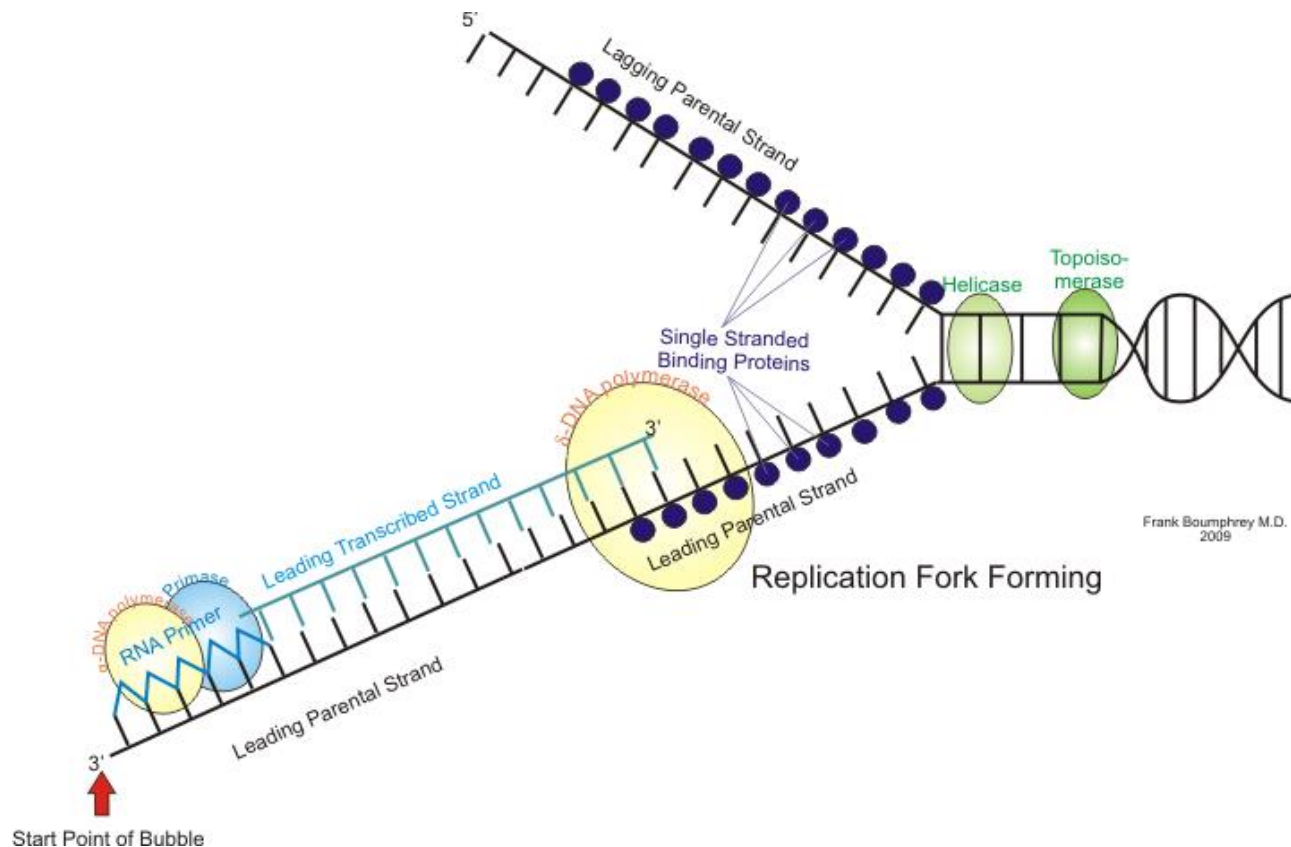


Elongation



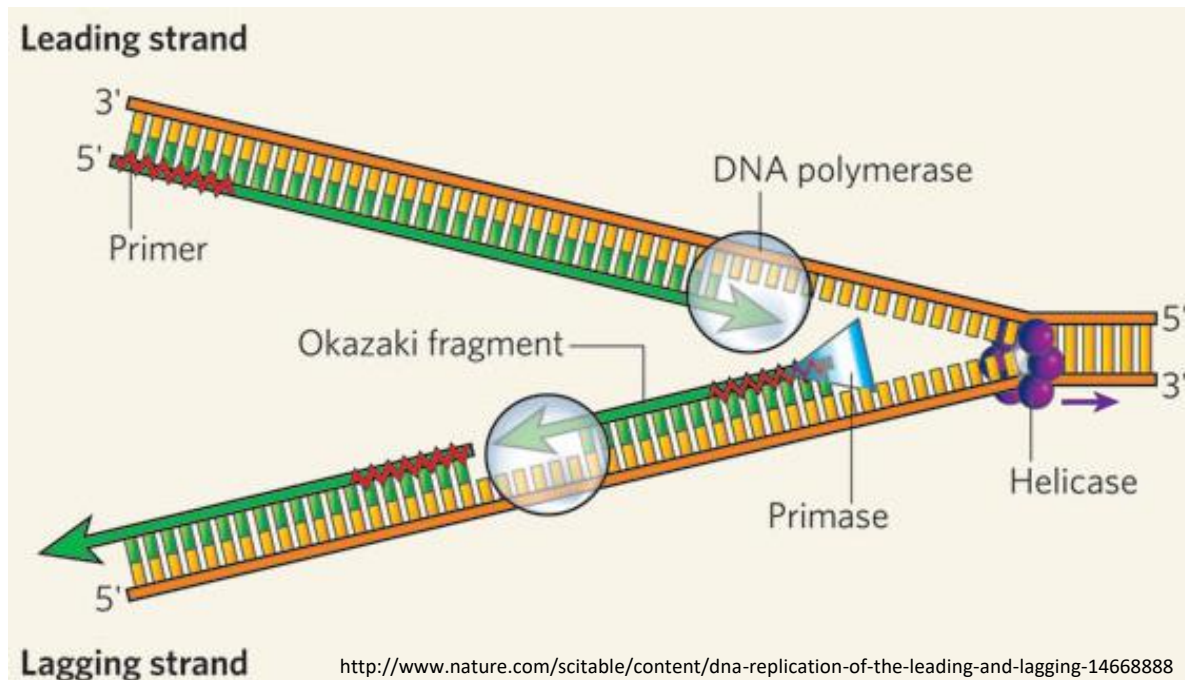
Leading Strand

- Replication is continuous
- Primase synthesises an RNA primer at the origin (provides free 3' OH group)
- DNA polymerase III binds primer and adds nucleotides in the 5' → 3' direction using the old strand as a template

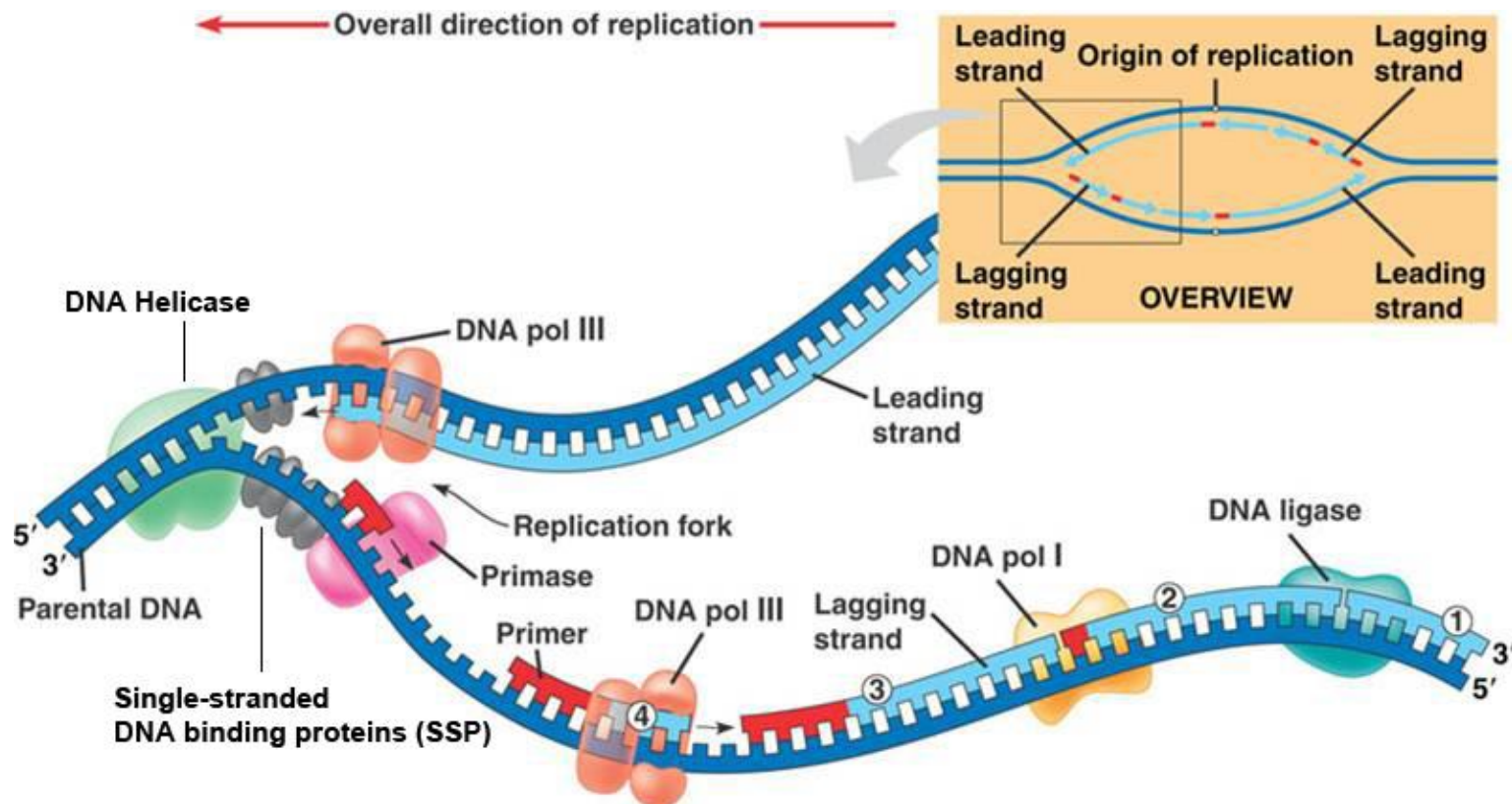


Lagging Strand

- Replication is discontinuous
- The template is $5' \rightarrow 3'$ = the wrong direction for continuous synthesis!
Synthesis cannot occur in the $3' \rightarrow 5'$ direction
- Primase synthesises an RNA primer near the origin
 - This provides the $3'OH$ groups for the addition of nucleotides - must occur again and again!
- DNA polymerase III
 - Binds to RNA primer and adds nucleotides in the $5' \rightarrow 3'$ direction, but only a limited number!



Lagging Strand

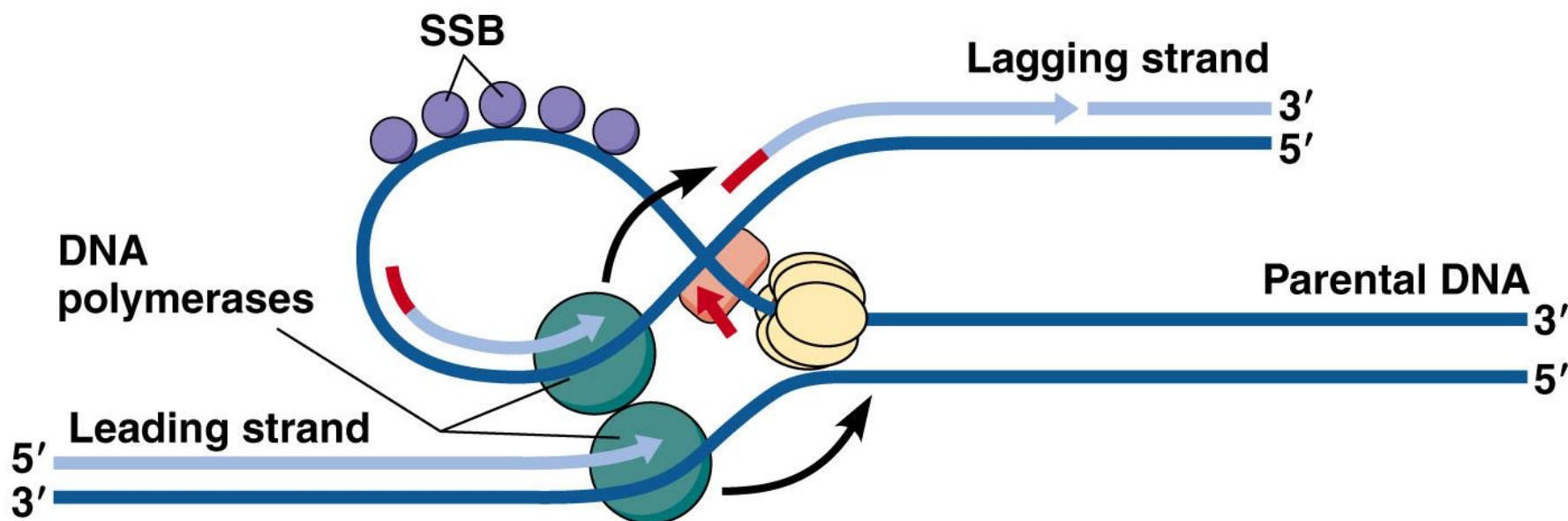


- **Remember:**

1. Instalments (Okazaki fragments)
2. Primase: synthesises multiple primers
3. DNA pol. (III): synthesises lagging strand
4. RNase H (or equivalent) digests RNA primer
5. DNA pol. (I): replaces digested RNA primer with DNA
6. DNA ligase: joins discontinuous fragments together

Lagging Strand – Problem of Antiparallel Nature

- Both template strands must be copied at the same place at the same time
- However, the replication machinery is faced with a direction problem due to the antiparallel nature of the template strands
- Work in prokaryotes suggests that this directionality problem can be solved by looping the lagging strand.
- This allows both polymerases to synthesis in the same direction at the same time

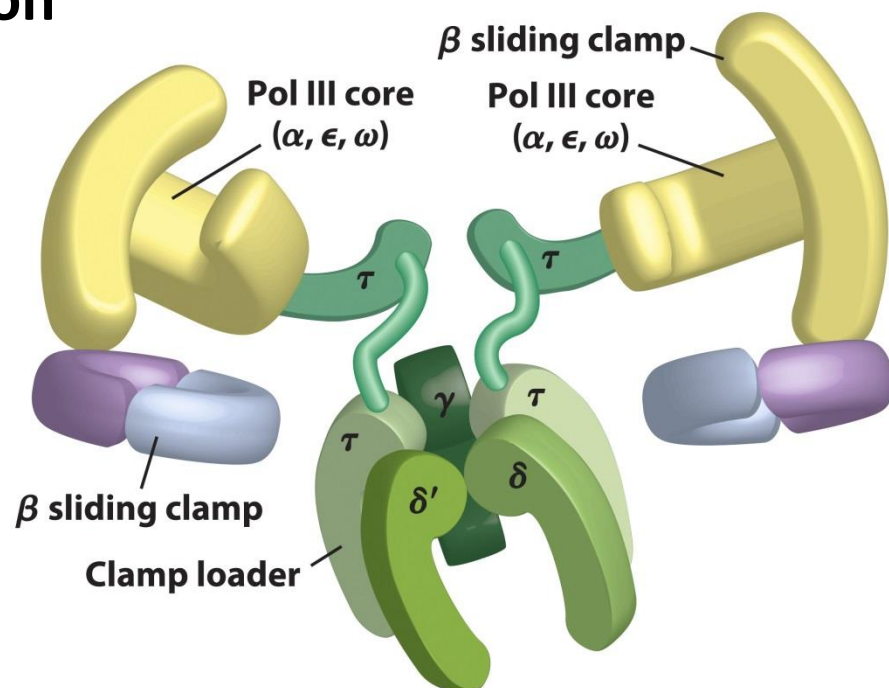
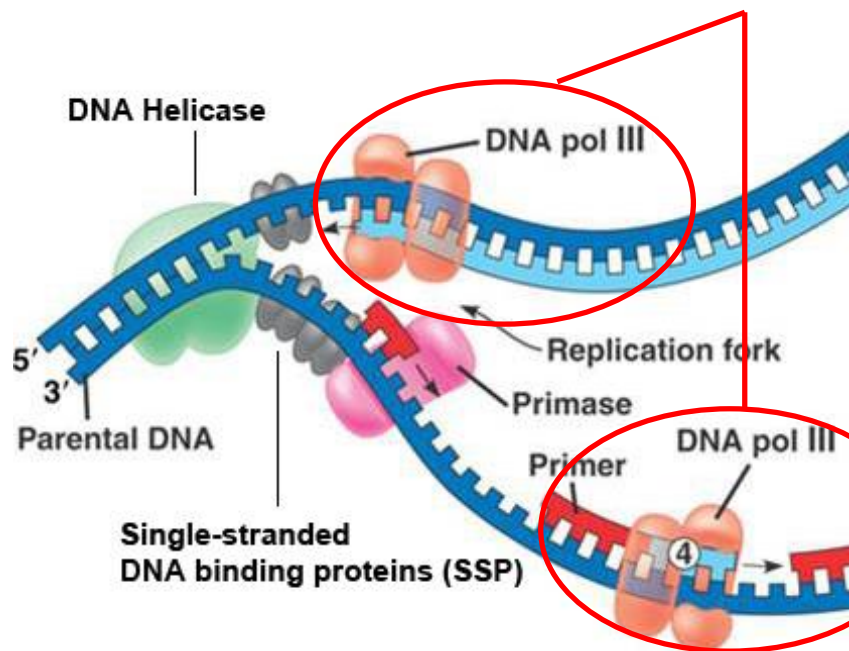


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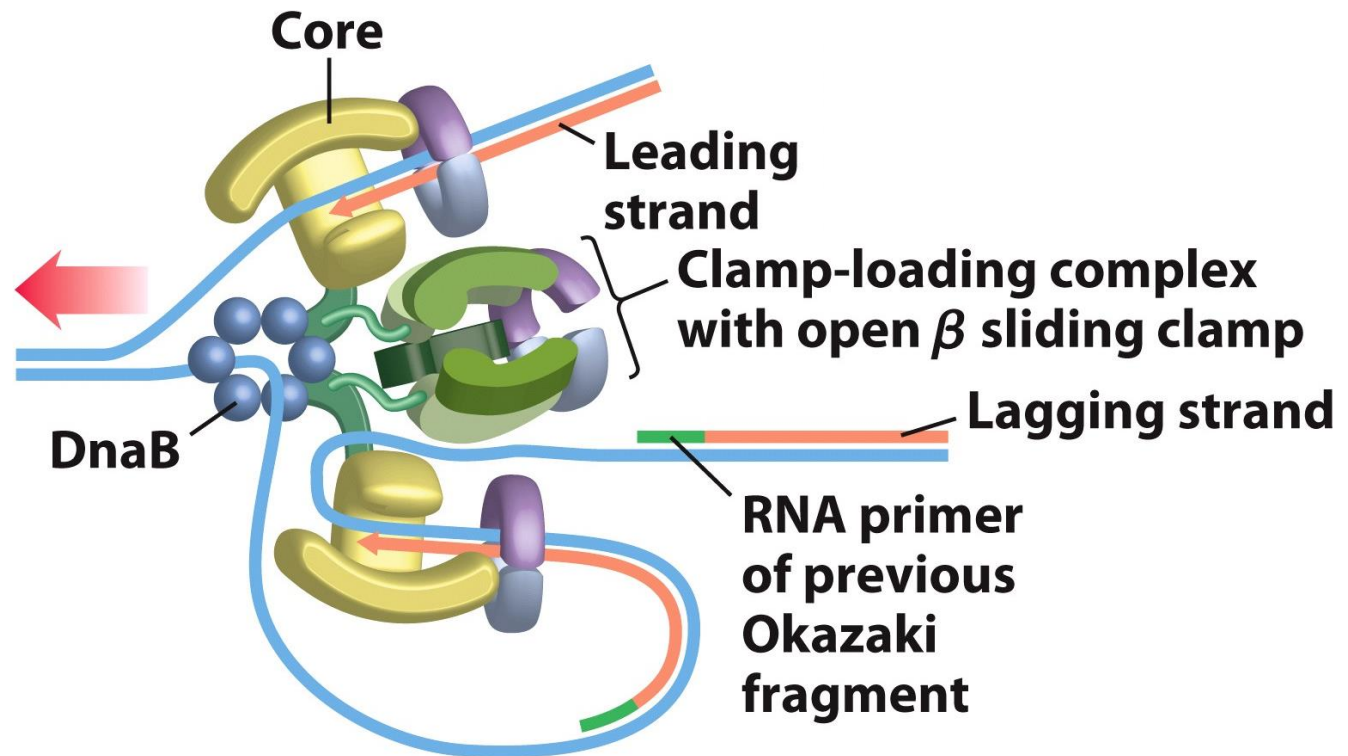
Structure of DNA Polymerase III

- Enzymatic (polymerase) activity occurs in dual core units (α, ϵ, θ)
- One core unit synthesises the leading strand and one the lagging strand
- Clamp loading component ($\tau\gamma\delta\delta'$)
- DNA Clamps (β subunits): increase processing rate, preventing dissociation from DNA strands

Oversimplification

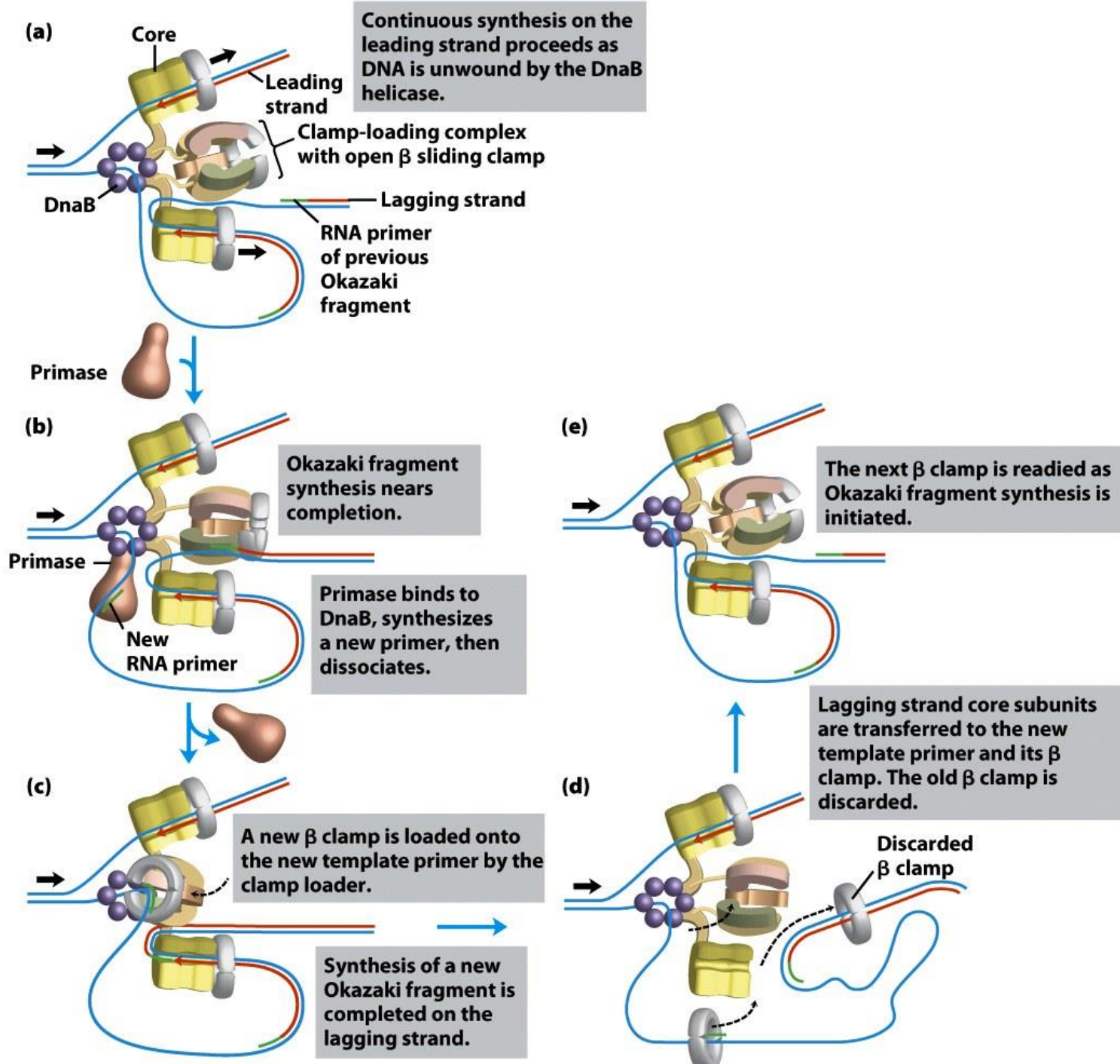


Lagging Strand



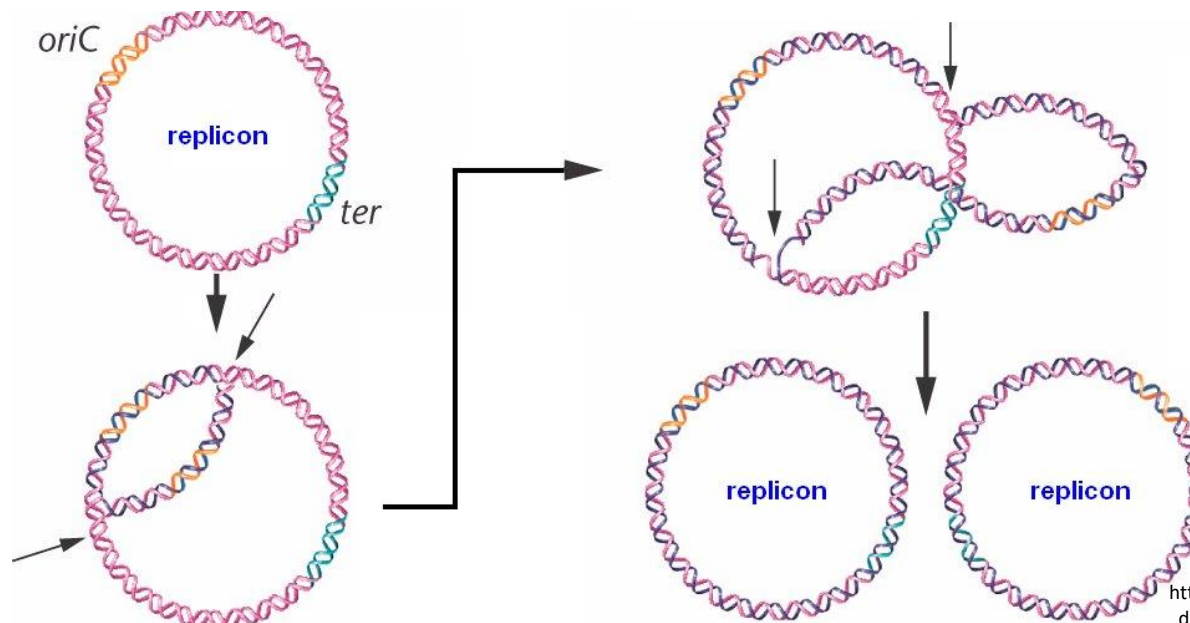
VIDEO

<http://www.dnatube.com/video/2335/Video-for-DNA-Replication>

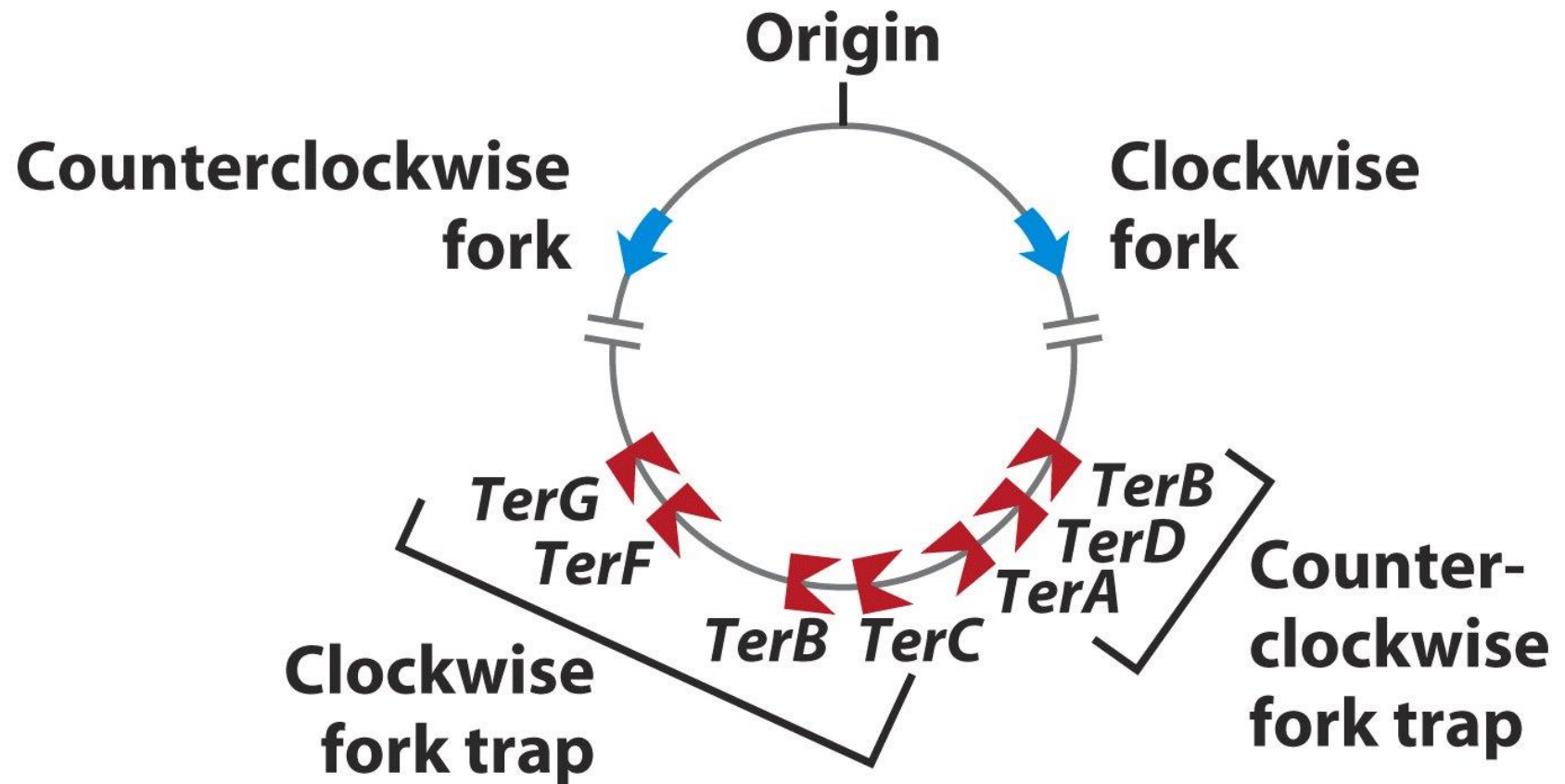


Termination

- The genome of *E. coli* is circular
- Eventually the 2 replication forks will meet
 - Then termination occurs
- Termination occurs in the terminus regions
 - 20 bp sequence of repeats (*Ter*)
 - Binding site for Tus (terminus utilisation substance) proteins
 - A Tus-Ter complex will trap one replication fork
 - The other fork will halt when it meets the first arrested fork

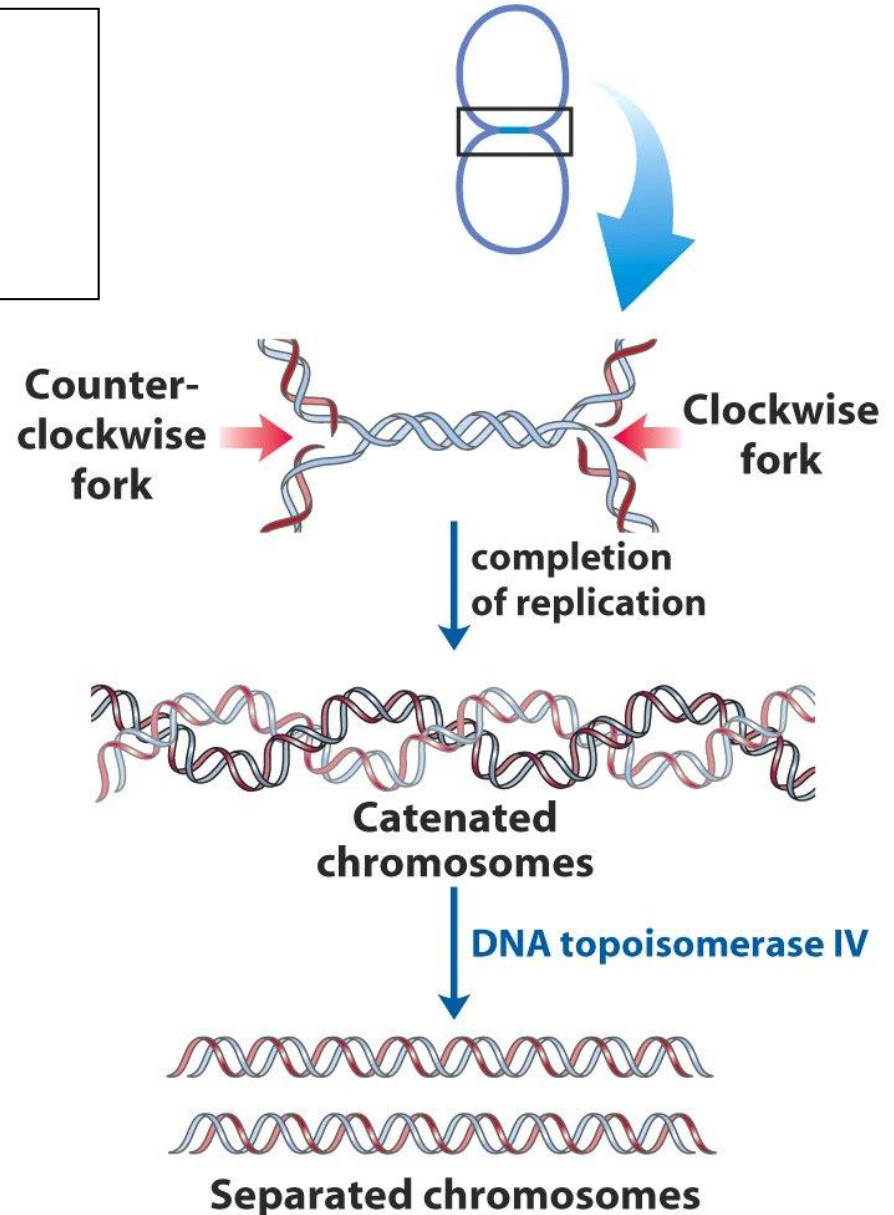


Termination



Termination

**DNA Topoisomerase IV
separates the 2 DNA
molecules**

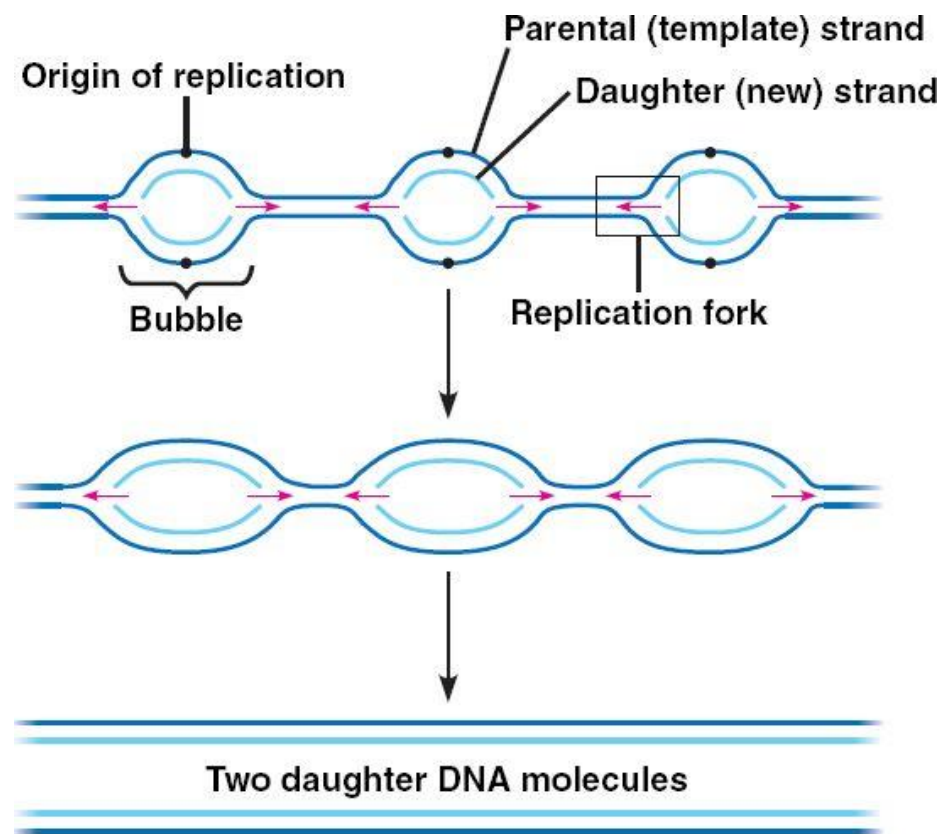


Ensuring Fidelity

- It is vital that the correct complementary sequence is generated in replication. Incorrect-pairing could permanently affect or eliminate the function of genes
- DNA polymerases can copy nucleotide sequences with incredible speed and fidelity (mistake = once every 10^9 to 10^{10} nucleotides added)
- Detection of incorrect base-pairing relies on
 - a) hydrogen bonding, and
 - b) geometry (only A=T and C≡G have correct geometry within the active site of the polymerase)
- Proof-reading provides fidelity:
 - DNA Polymerase has a $3' \rightarrow 5'$ exonuclease activity (double checks and removes mismatch mutations)
 - Incorrect insertion of a nucleotide inhibits further elongation, allowing $3' \rightarrow 5'$ exonuclease activity to take place.

What about Eukaryotes?

- Eukaryotes:
 - More DNA
 - Packaged into nucleoprotein complexes
 - Greater complexity, highly regulated to coordinate with the cell cycle (occurs only once per cycle)
 - Essential features of replication are the same
 - Multiple origins of replication
 - Initiation at the origin recognition complex (ORC)
 - Polymerases are named differently
 - Termination involves telomeres



<http://bio1151.nicerweb.com/Locked/media/ch16/init.html>

Test your Knowledge

When a DNA molecule is described as replicating bi-directionally, that means that it has two:

- a) chains
- b) independently replicating segments
- c) origins
- d) replication forks
- e) termination points

Test your Knowledge

At the replication forks in *E. coli*:

- a) DNA helicases make endonucleolytic cuts in DNA.
- b) DNA primers are degraded by exonucleases.
- c) DNA topoisomerases make endonucleolytic cuts in DNA.
- d) RNA primers are removed by primase.
- e) RNA primers are synthesized by primase.

Test your Knowledge

An Okazaki fragment is a:

- a) Fragment of DNA resulting from endonuclease action.
- b) Fragment of RNA that is a subunit of the 30S ribosome.
- c) Piece of DNA that is synthesized in the $3' \rightarrow 5'$ direction.
- d) Segment of DNA that is an intermediate in the synthesis of the lagging strand.
- e) Segment of mRNA synthesized by RNA polymerase.

What's Next?

- ✓ • Topic 1 – Foundations in Biochemistry
- ✓ • Topic 2 – Amino Acids
- ✓ • Topic 3 – Proteins
- ✓ • Topic 4 – Lipids and Membranes

- ✓ • Topic 5 – Signal Transduction
- ✓ • Topic 6 – Enzymes
- ✓ • Topic 7 – Nucleotides and Nucleic Acids
- ✓ • Topic 7 – DNA Replication
 - Topic 8 – Transcription
 - Topic 9 – Translation

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