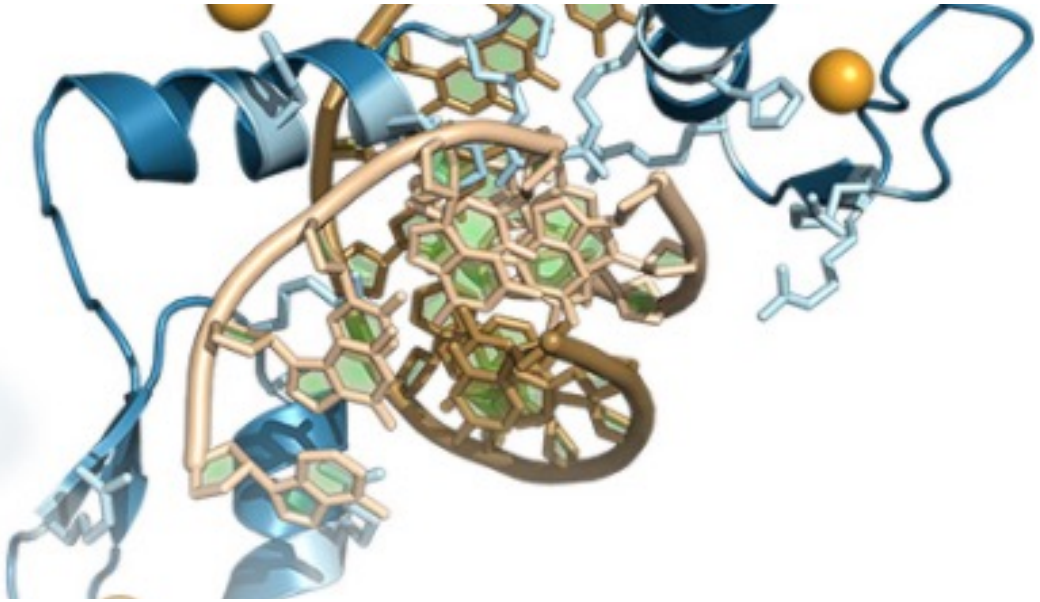
© COPYRIGHT NOTICE

CELS191 LECTURE MATERIAL

Department of Biochemistry

Te Tari Matū Koiora



Dr Annika Bokor

Biochemistry

annika.bokor@otago.ac.nz

Research & Interests:

Biochemistry, Genetics & Forensics

CELS191 2025

Molecular Biology & Genetics

Lecture 13

DNA Replication

Dr Annika Bokor
Te Tari Matū Koiora | Department of Biochemistry

Lecture 13 Objectives

After you have revised this lecture you should be able to:

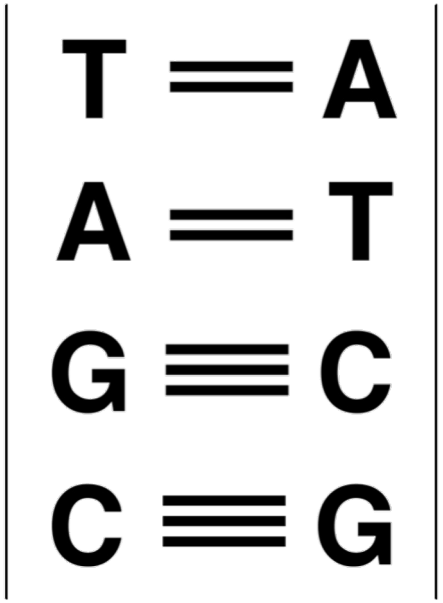
- Describe the mechanism of DNA replication and the specific functions of all the molecules required.
- Describe how errors in the DNA sequence can be corrected and explain why this is important.

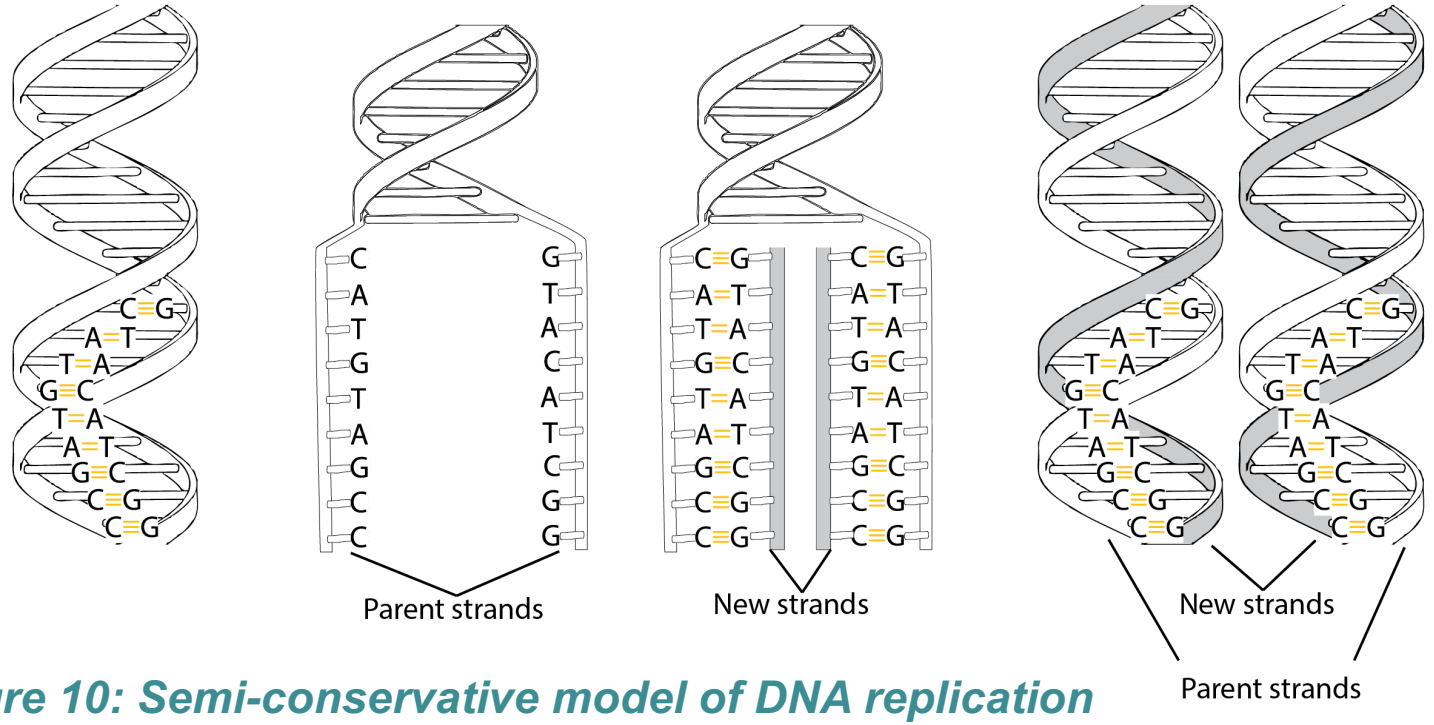
Context Slide

CELS191 Supplied Image

"It has not escaped our notice that the specific pairing we have postulated immediately suggests a possible copying mechanism for the genetic material "

(Nature, April 25 1953)

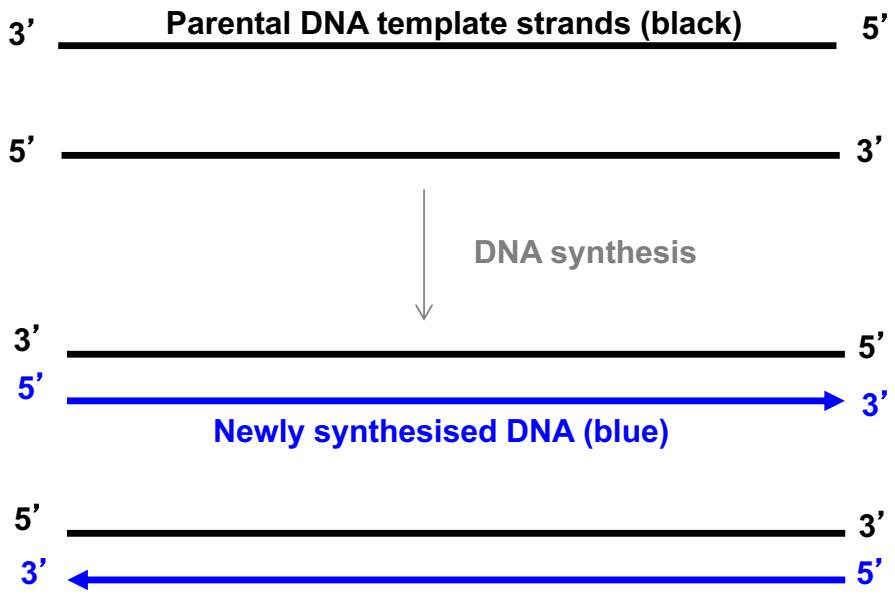




Lecture 10: Semi-conservative model of DNA replication

Direction of DNA Synthesis:

- DNA (or RNA) is **ALWAYS synthesised** in the 5' → 3' direction (remember the 3rd carbon OH group!)
- Thus, the parental **template** strands are said to **be/run** in the 3' → 5' direction



Eukaryotic DNA Replication

- Multiple large linear chromosomes

(23 pairs in Humans)

- Multiple origins of replication (ori)
- Bidirectional

Teal:
Parental DNA strands

Grey:
Newly synthesised (daughter) DNA strands

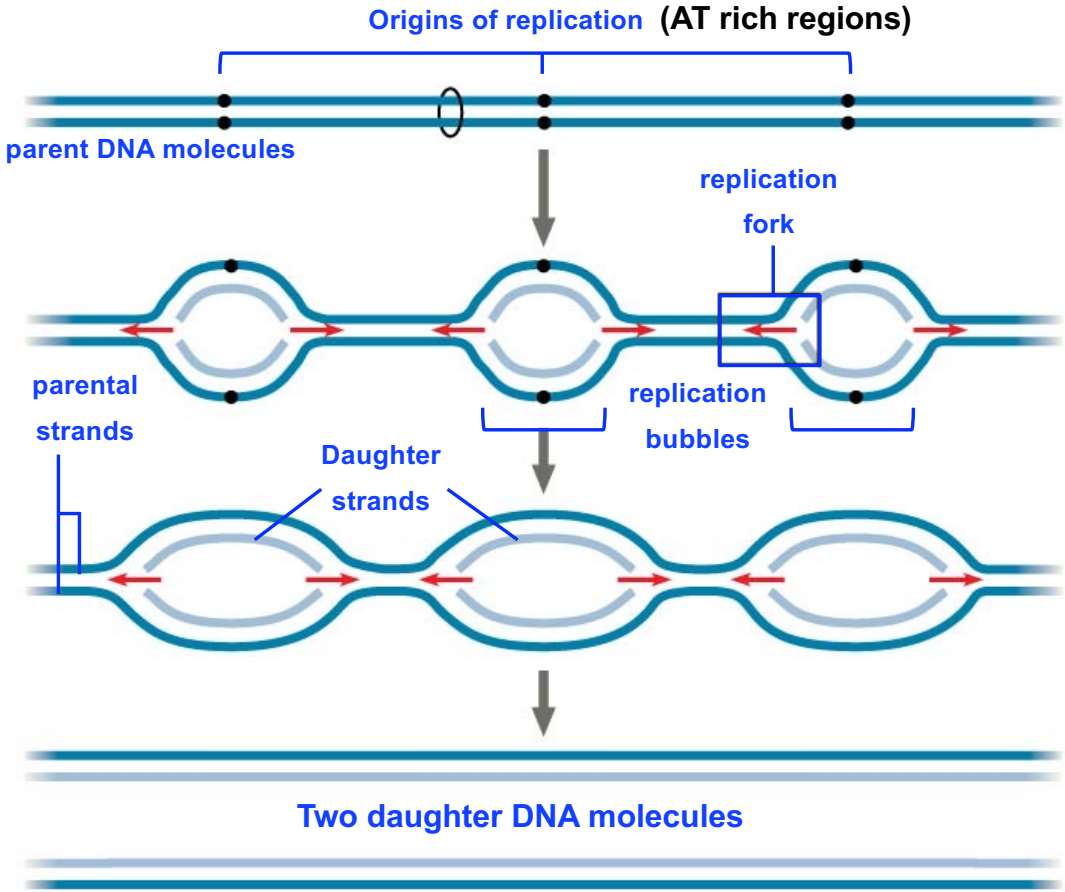
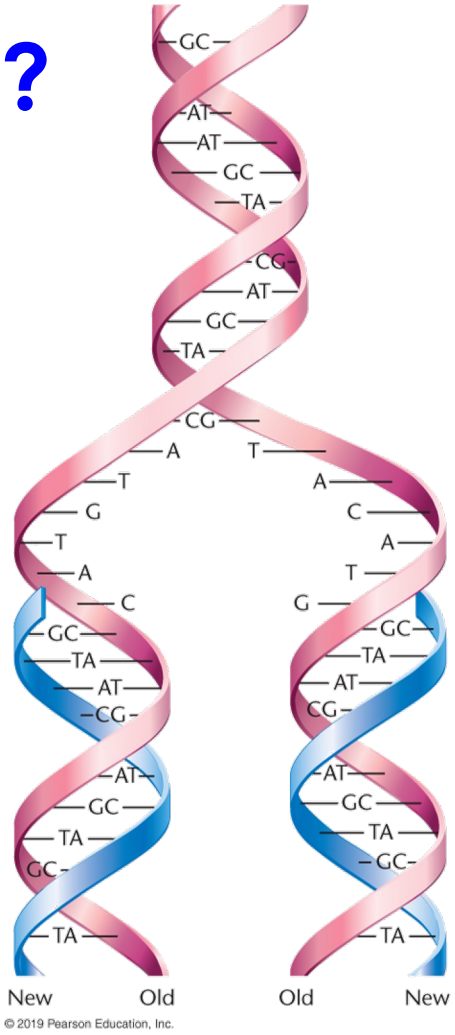


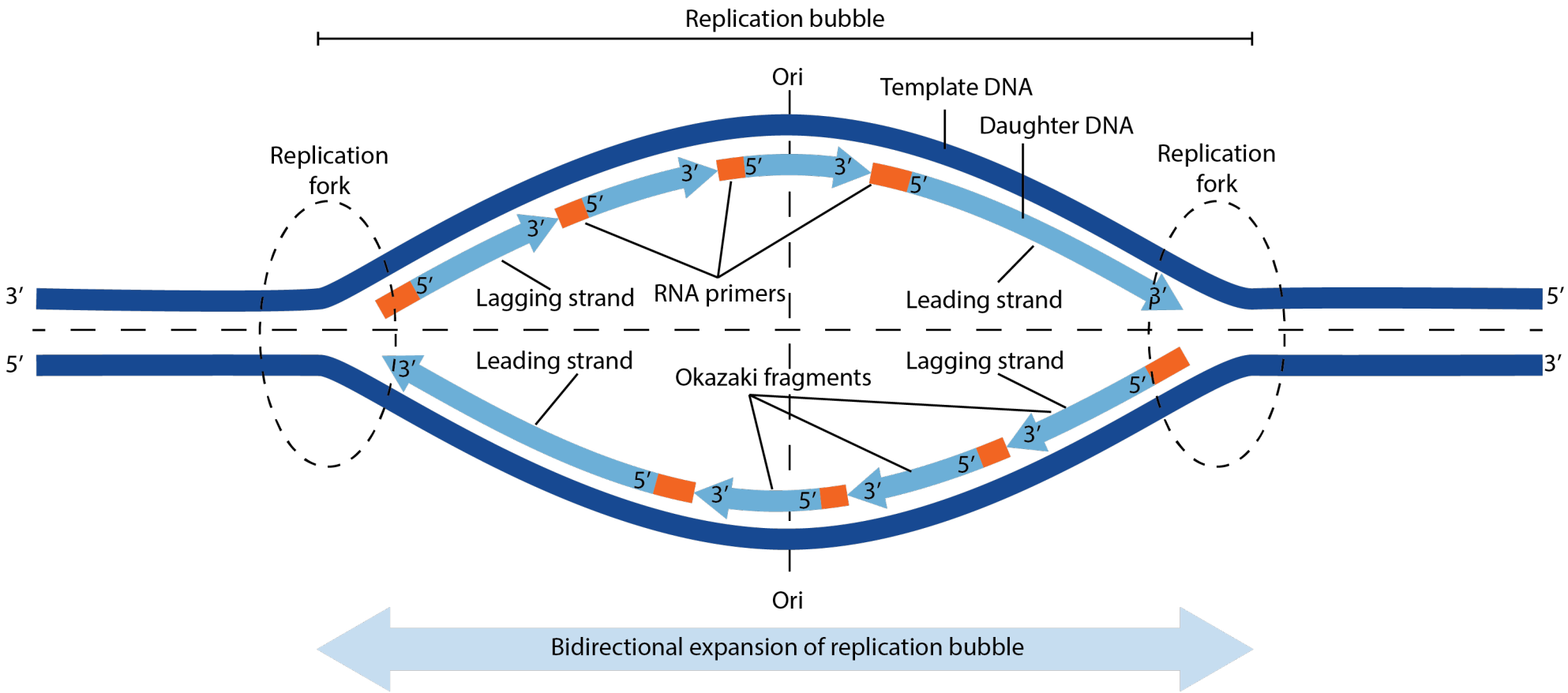
Figure 11.1: Concepts of Genetics Klug et al 12ed

What is needed to make a DNA copy?

- Progressive addition of new nucleotides (A, C, T or G)
- A starting point for nucleotide addition
- Unwinding of the helical double-stranded DNA (to give two parental templates)
- Release of tension generated by unwinding the DNA helix
- Prevention of unwound double-stranded helical DNA, i.e. single-stranded DNA, from reforming and to protect it
- Joining of ends of newly synthesised fragments together (lagging as well as leading strands)



Replication is Semi-discontinuous

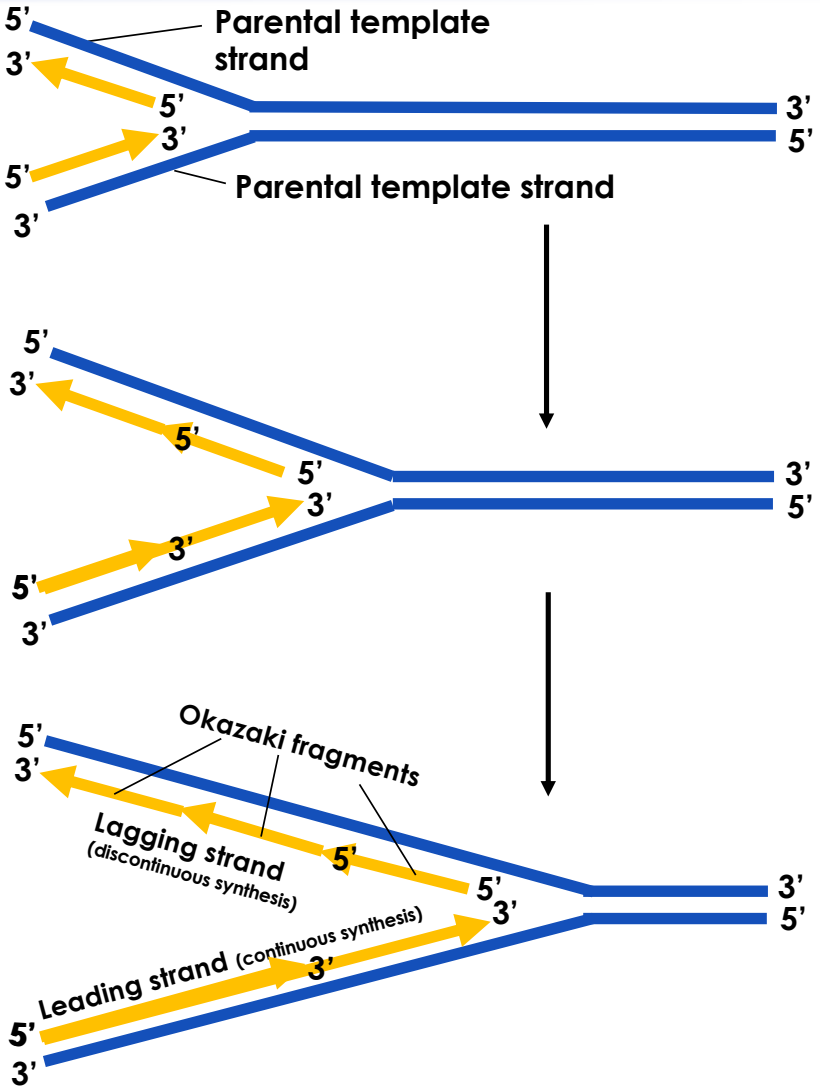


Replication is Semi-discontinuous

Leading Strand: Continuously synthesised in its 5' → 3' direction

Lagging Strand: Discontinuously synthesised in its 5' → 3' direction as Okazaki fragments

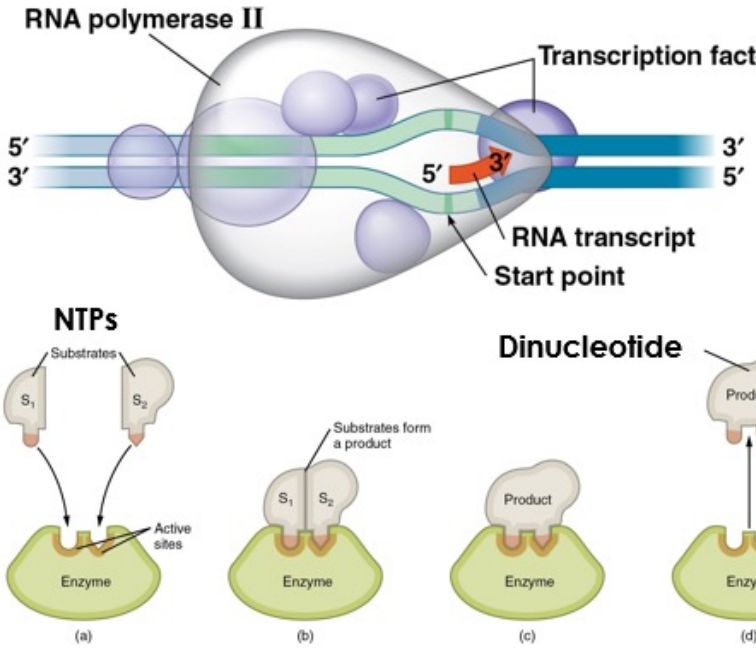
Direction of DNA synthesis is in towards the replication fork



Images:
Honeydew: https://commons.wikimedia.org/wiki/Category:Honeydew_melon
Zipper: https://www.freeiconspng.com/img/48761
Enzyme: https://philschatz.com/anatomy-book/contents/m46008.html

Lecture 11: Initiation of RNA synthesis

RNA pol II recruits **Helicase** – an enzyme that 'unzips' DNA by breaking H-bonds between the DNA base components. Helicase binds to the AT-rich region of the promotor to start 'unzipping'.



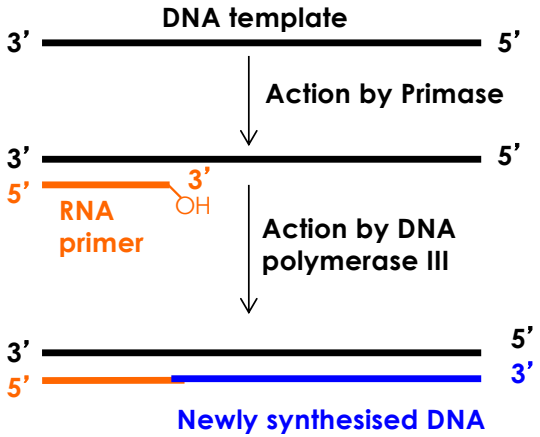
The two DNA strands separate, and RNA pol II starts mRNA synthesis **without the need of a primer.**

RNA pol II has a **primase** activity

Figure 11.1: Concepts of Genetics Klug et al 12ed

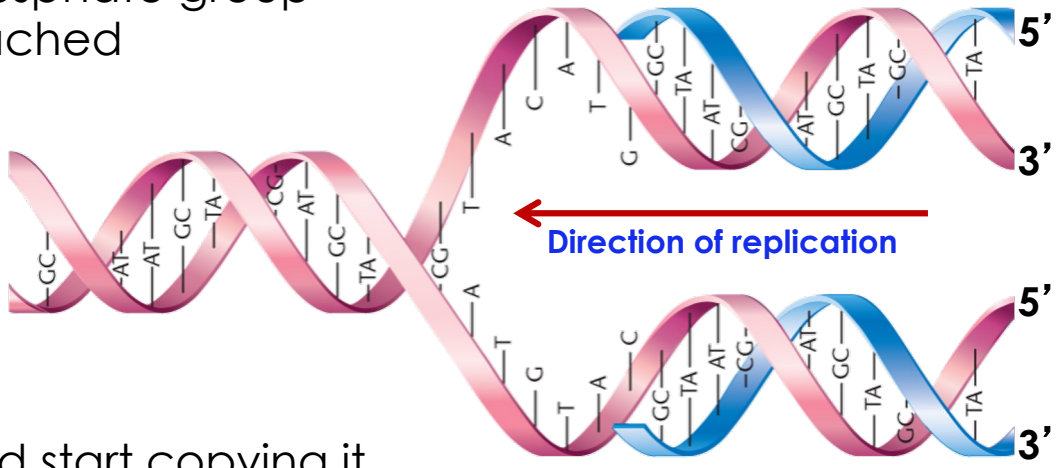
Primase

- An enzyme, a type of RNA polymerase, that makes an RNA primer (***see lecture 11***)
- Primer will act as a starting point for DNA polymerization

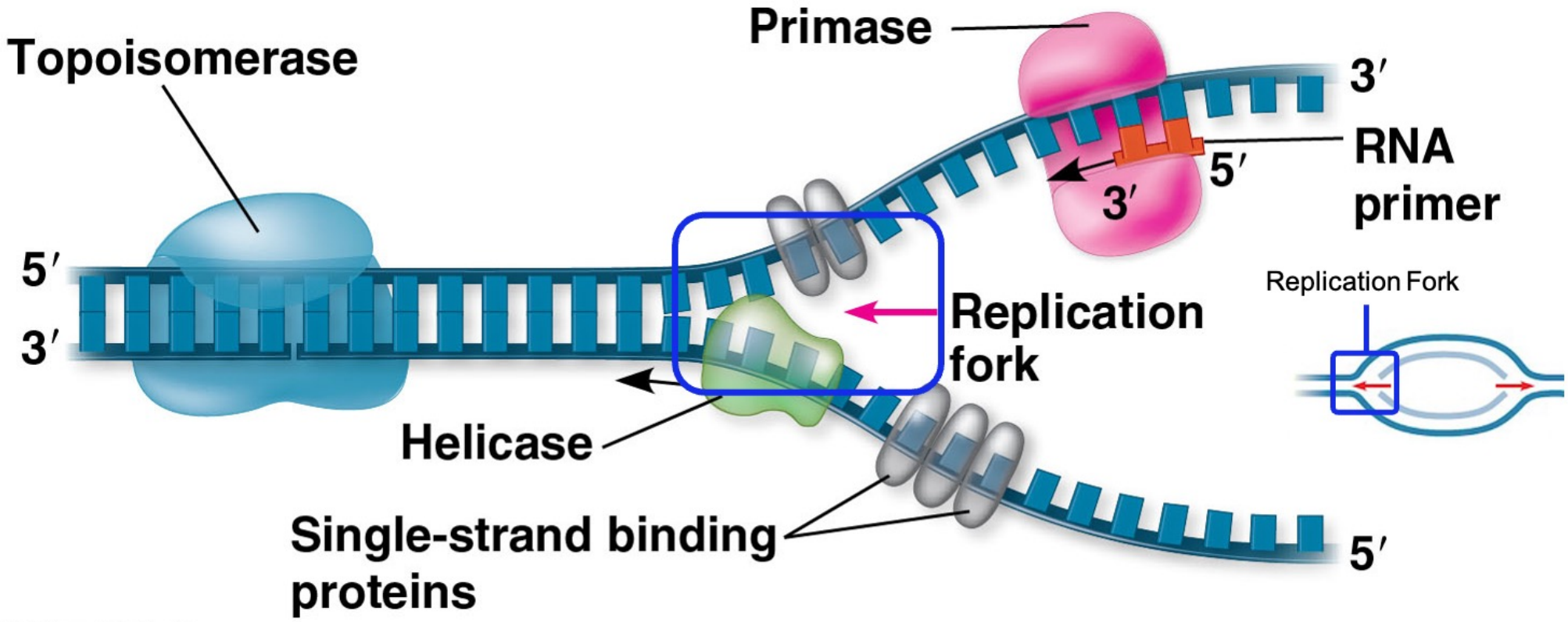


DNA Polymerase III *(Pol III)*

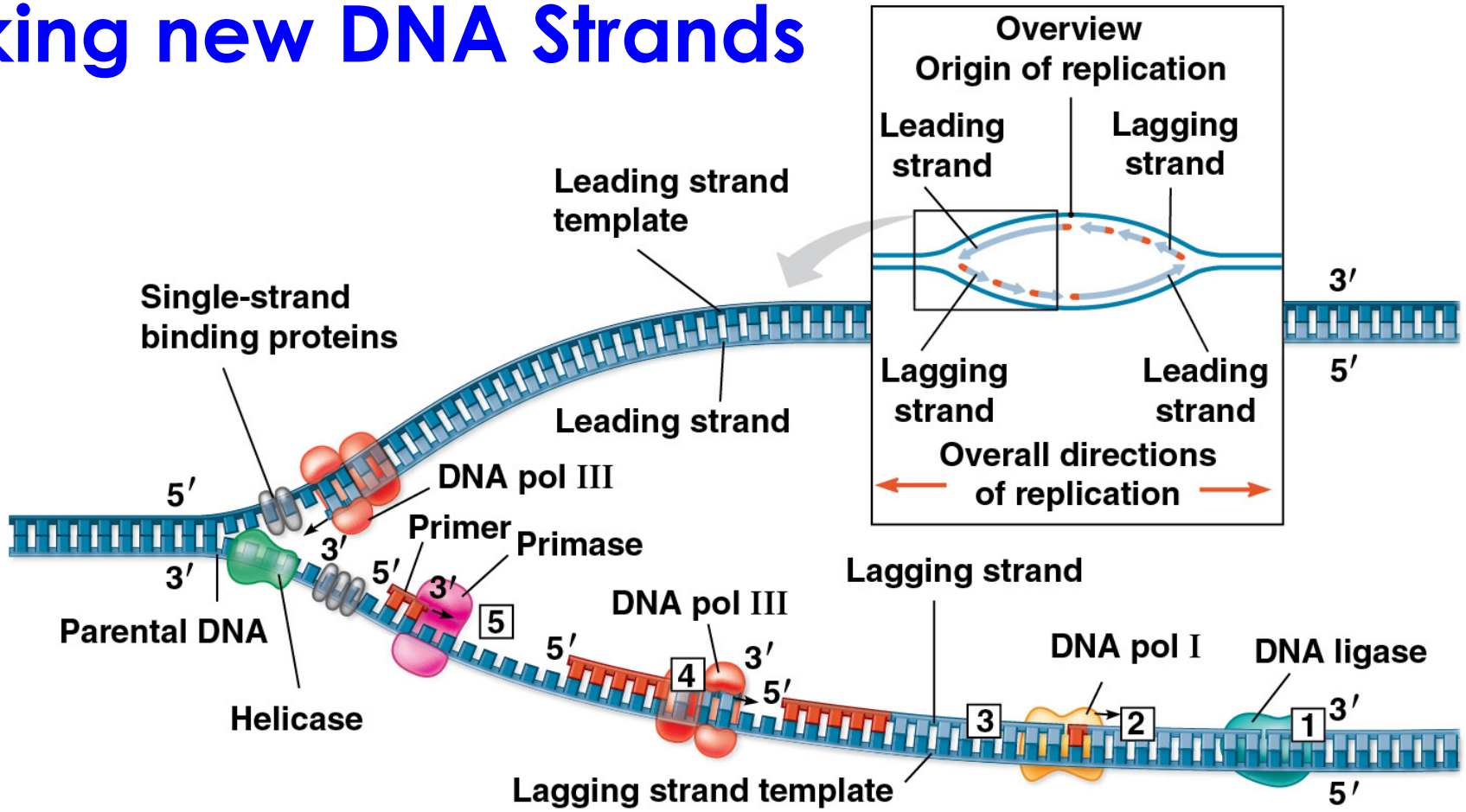
- Needs an OH group onto which the phosphate group of the incoming nucleotide can be attached
- Only makes DNA in the 5' → 3' direction
- Enzyme that synthesises a new DNA strand by adding nucleotides complementary to the parental template strands
- Cannot bind to single stranded DNA and start copying it



Initiating DNA Replication



Making new DNA Strands

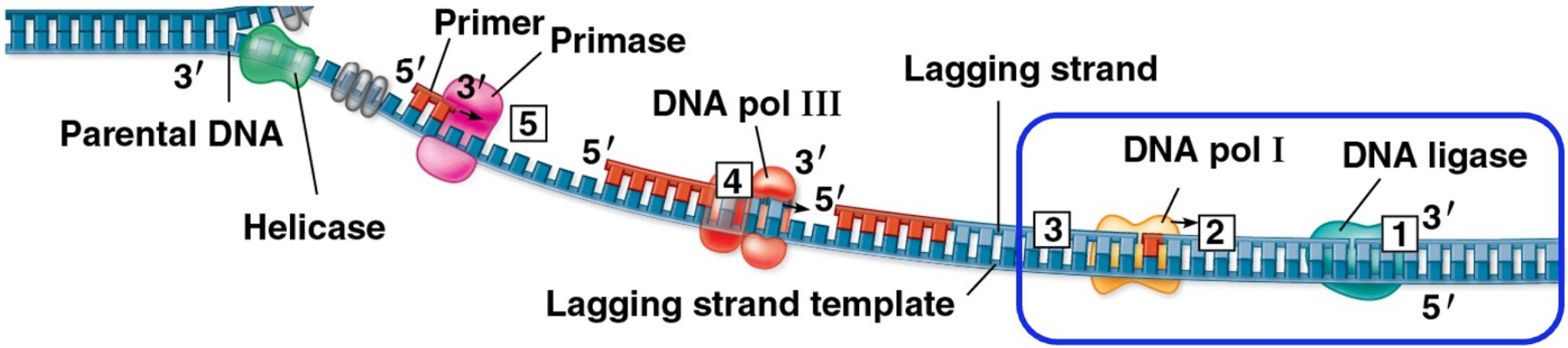


DNA Polymerase I

Two activities: Removes RNA primers (RNase H) and fills the gap with DNA nucleotides (DNA polymerase)

DNA Ligase

Joins the newly synthesized Okazaki fragments together (creates phosphodiester bonds)



DNA Pol I carries out two activities:

1. RNase Activity:

- RNase H is an endonuclease enzyme that recognises DNA:RNA hybrids and degrades the RNA part

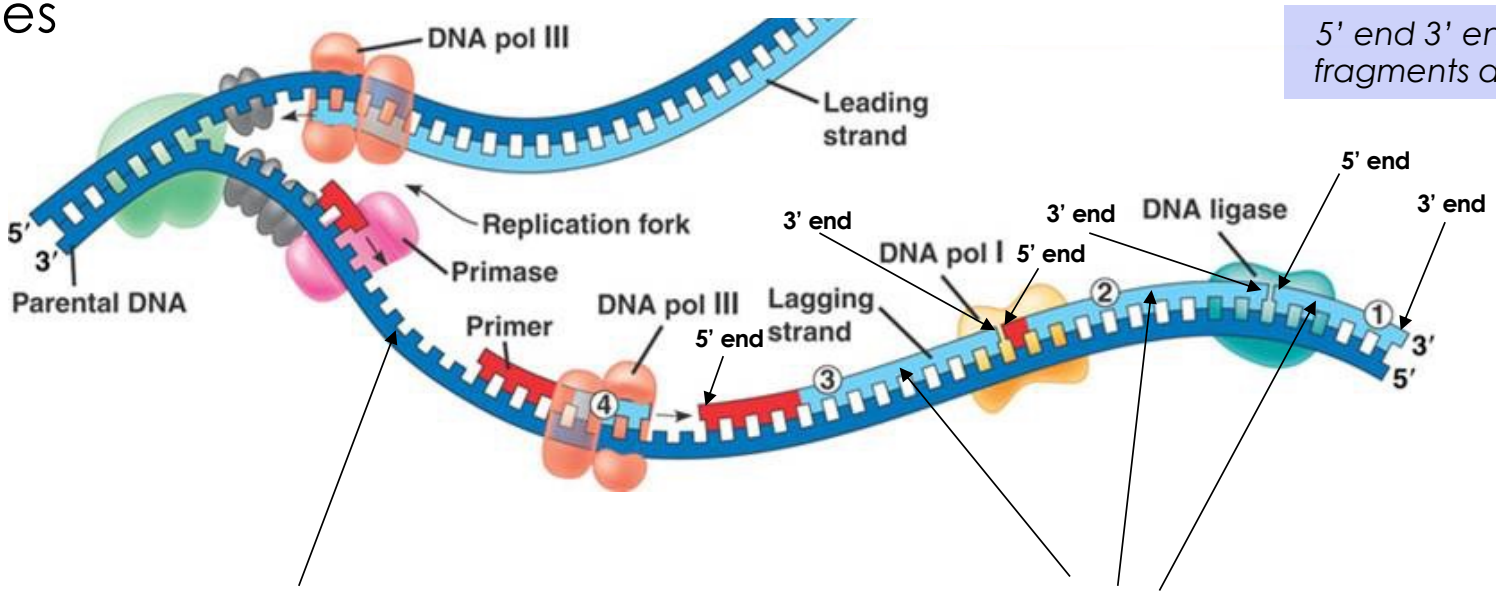
2. DNA Polymerase Activity:

- Synthesises DNA by adding nucleotides (complementary to the parental DNA template of the lagging strand)

DNA ligase

Joins newly synthesised Okazaki fragments together (creates phosphodiester bonds), once the RNA primers have been removed and replaced by DNA nucleotides

5' end 3' ends of the Okazaki fragments are labelled below



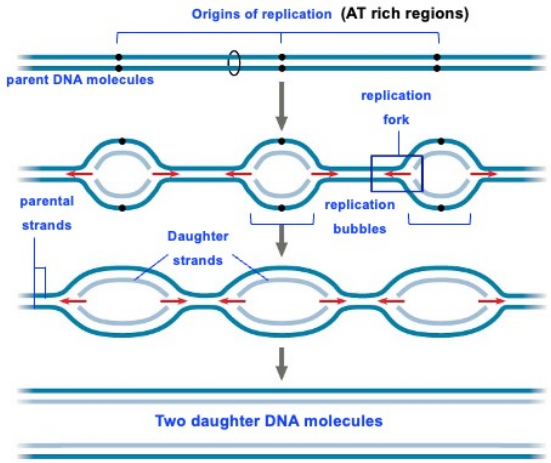
Template strand (dark blue)

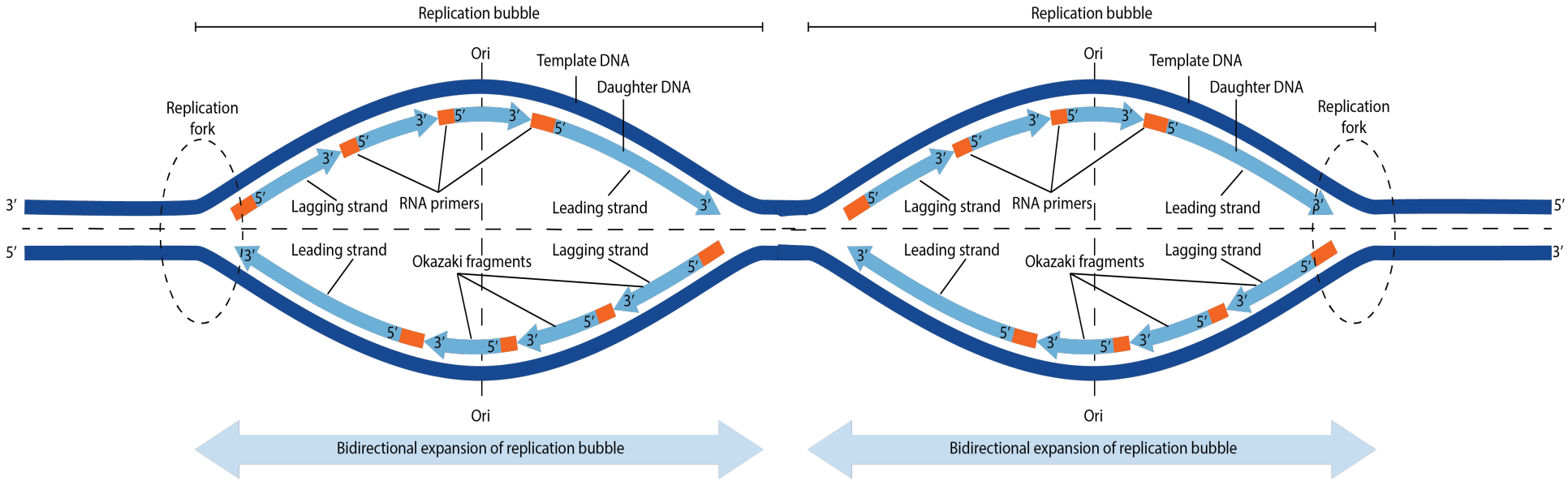
Okazaki fragments (light blue)

CELS191 Supplied Image & Modified Textbook Figure 16.13b

DNA ligase

Not only joins together the lagging strand (Okazaki) fragments together, but also the the newly synthesised fragments from the multiple replication bubbles, including the leading strands



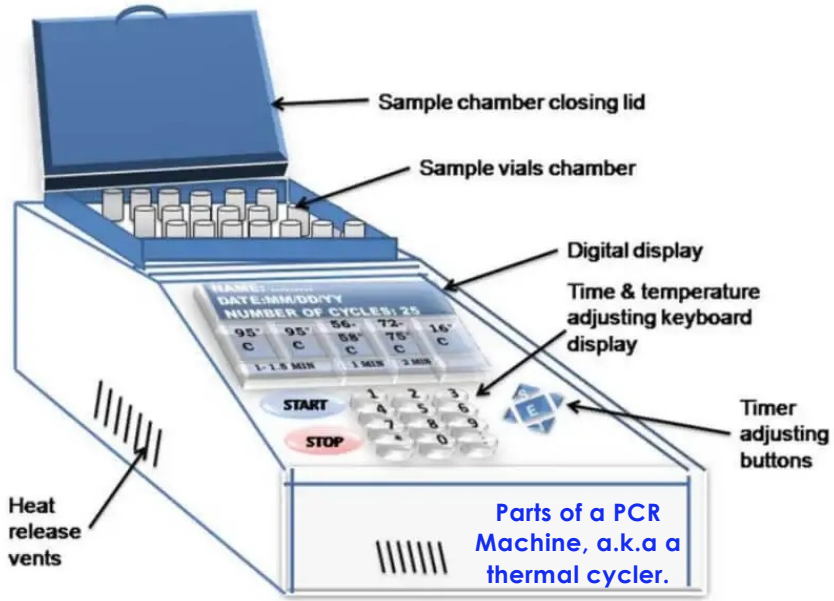


What is needed to make a DNA copy?

- Progressive addition of new nucleotides (A, C, T or G)
  DNA polymerase III
- A starting point for nucleotide addition
  Primase enzyme makes RNA primer
- Unwinding of the helical double-stranded DNA to give two parental templates
  Helicase
- Release of tension generated by unwinding the DNA helix
  Topoisomerase nicks and rejoins DNA strands
- Prevention of unwound double-stranded helical DNA, i.e. single-stranded DNA, from reforming and to protect it from degradation
  Single-stranded DNA binding protein
- Removes RNA primer and fills gap with DNA nucleotides
  DNA polymerase I (RNase H activity removes RNA and DNA polymerase adds the DNA nucleotides)
- Joining of ends of newly synthesised fragments together (lagging as well as leading strands, within and between replication bubbles)
  DNA ligase

Image: Aakanchha Jain et al. 2020.

Polymerase Chain Reaction (PCR)



- *In vitro* DNA replication (DNA replication in a test tube)
- A laboratory technique used to make millions to billions of copies of **a particular section of DNA** from a very small original amount, which can then be studied in greater detail.

More on PCR in Lecture 27

Repair of DNA errors

When can DNA errors be repaired?

1. DURING replication

(using an **EXOnuclease**)

2. AFTER replication

(using an **ENDOnuclease**)

Repair of DNA errors **DURING** DNA Replication

DNA replication shows high accuracy:

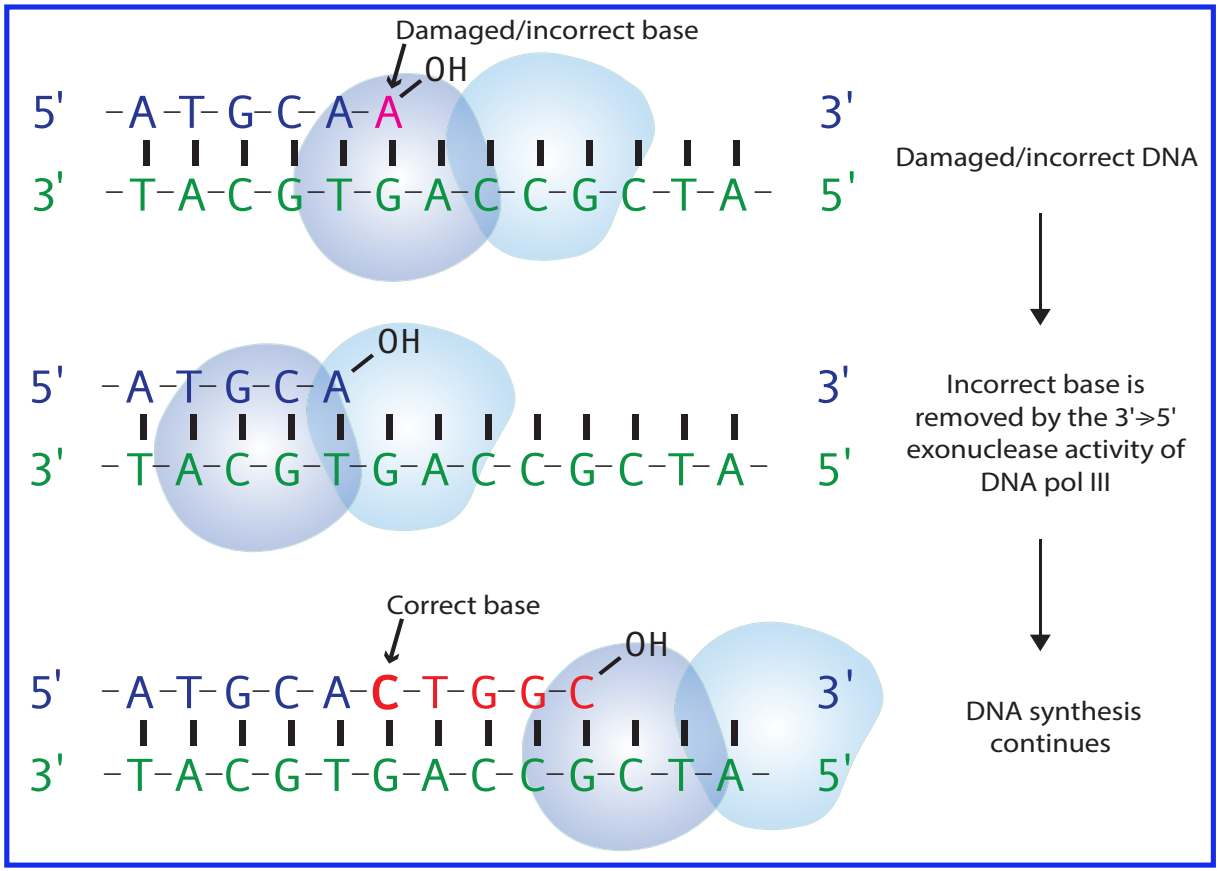
- DNA pol III has a replication error rate of 1 in 10^8 - 10^{10} base-pairs replicated

DNA pol III has a proofreading mechanism:

- Checks the newly inserted nucleotide bases against the template

These types of incorrect bases are removed by a 3' to 5' EXOnuclease activity of DNA Pol III

3' to 5' exonuclease activity of DNA pol III removes incorrect bases DURING DNA synthesis



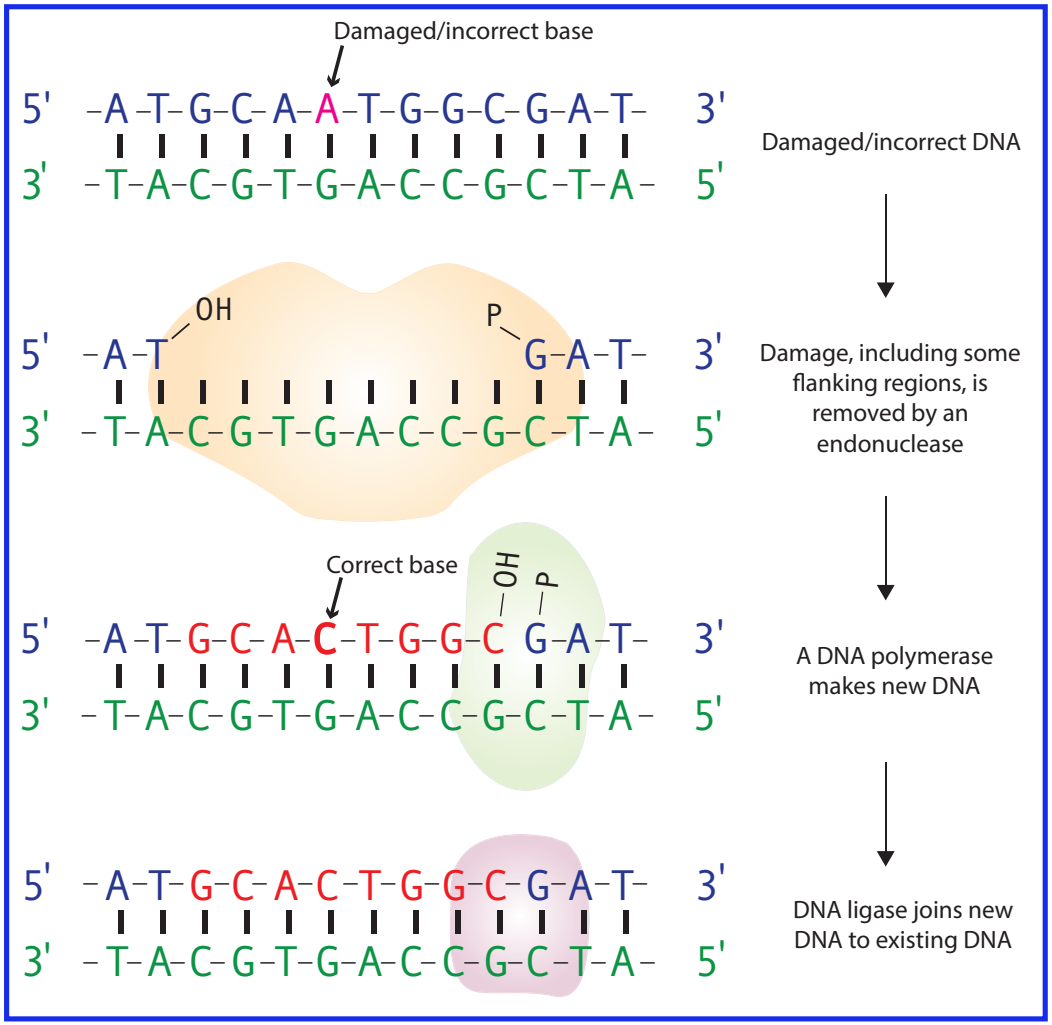
Repair of DNA errors **AFTER** DNA Replication

A variety of things can cause DNA damage or errors, e.g.:

- Incorrectly inserted bases are not corrected by DNA pol III
- Radiation damage (e.g. UV)
- Chemical modifications of bases (natural & chemical causes)

These types of incorrect or damaged nucleotide bases are removed by an ENDOnuclease

There are various types of DNA error and damage repair systems, but the general idea behind correcting DNA errors **AFTER** replication is:

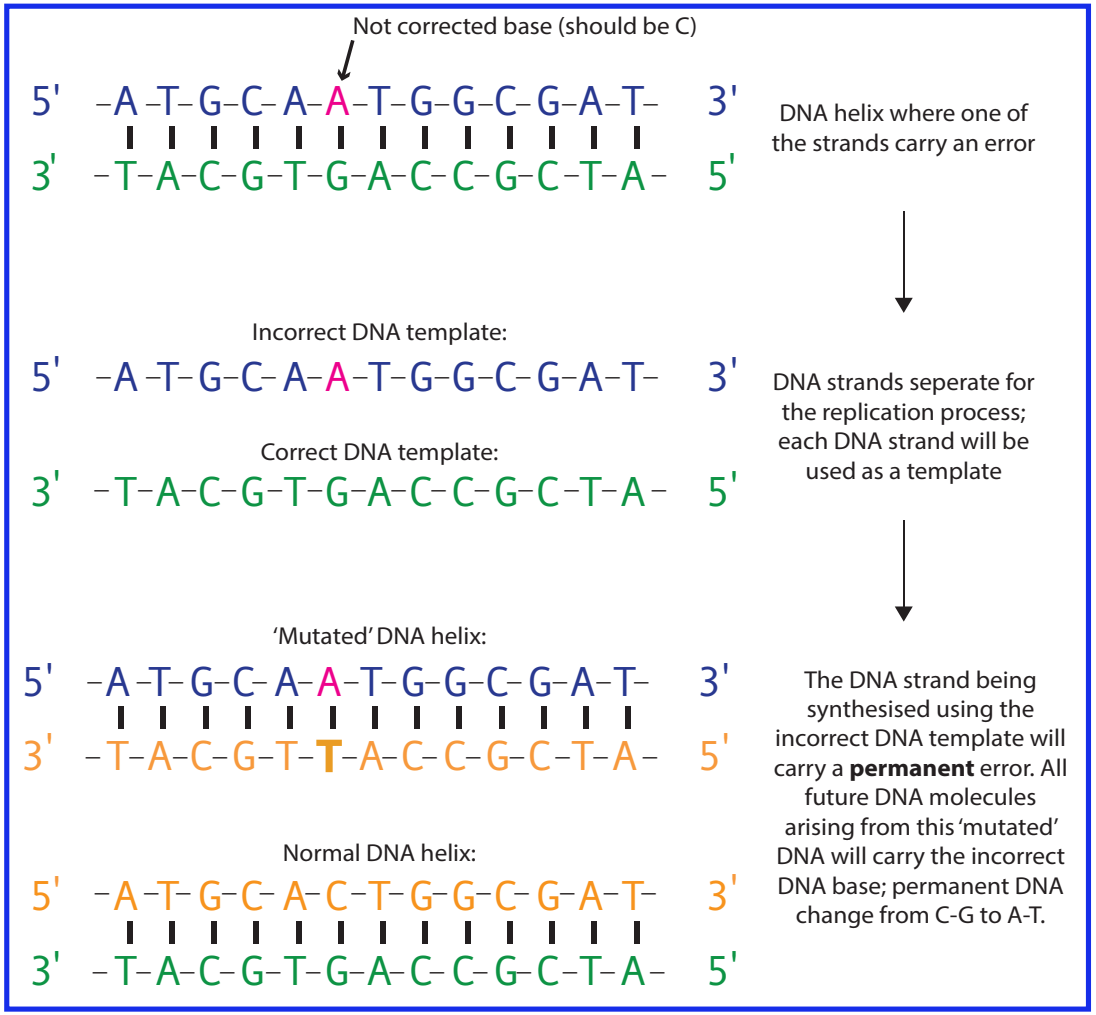


Importance of correcting DNA errors

If not corrected, the DNA error becomes part of the DNA template

➔ permanent DNA change

i.e. DNA damage /mutation



Lecture 13 Summary

- DNA replication is semi-discontinuous, where the leading strand is synthesized continuously and the lagging strand discontinuously.
- Both DNA strands are used as template strands in DNA replication.
- Eukaryotic chromosomes are replicated through a large number of replication bubbles, which eventually join up.
- Many enzymes are involved in DNA replication.
- Exonucleases and endonucleases are involved in correcting DNA errors that have arisen during and after DNA replication respectively.

Objective-Based Questions

- Describe the function and importance of 7 proteins (all are enzymes) that are involved in DNA replication.
- Why does the lagging strand need to be synthesised as smaller fragments, and what are these smaller fragments called?
- Why is the primer made by the primase enzyme in DNA replication removed and replaced? (Note that the primer is not removed in transcription)
- What 'job' can an enzyme with a 3' to 5' exonuclease activity perform?
- Explain how permanent DNA errors arise.

CELS191 2025

Molecular Biology & Genetics

Lecture 13

DNA Replication

Dr Annika Bokor
Te Tari Matū Koiora | Department of Biochemistry