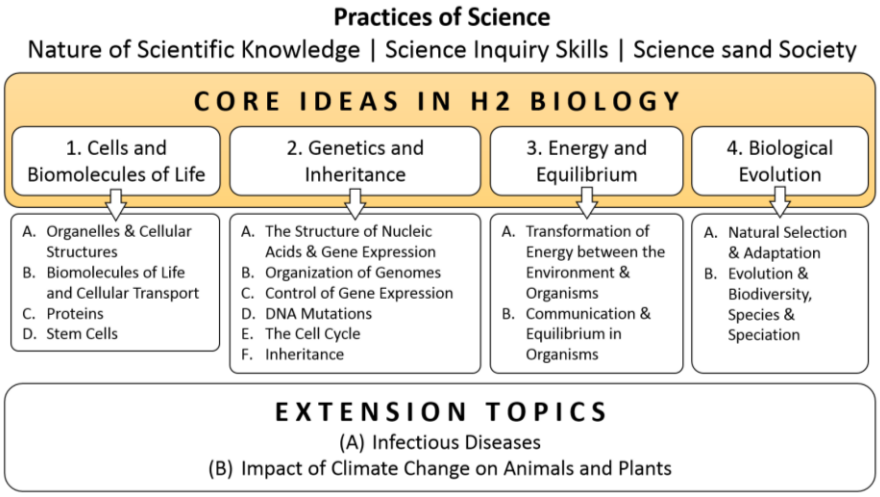
Tampines Meridian Junior College
JC1 H2/9744 Biology 2023

Complete the pre-lecture exercise on pg 5

Core Idea 2A
5. Genetics & Inheritance (II) – DNA Replication & Gene Expression



SYLLABUS OVERVIEW		
No.	**Overarching Idea**	**Topics**
1	**Core Idea 1** The Cell and Biomolecules of Life	Cell – The Basic Unit of Life
2		Biomolecules of Life and Cellular Transport
3	**Core Idea 3** Energy and Equilibrium	Transformation of Energy – Photosynthesis and Cellular Respiration
4	**Core Idea 2** Genetics and Inheritance	Genetics and Inheritance (I) – The Cell Cycle
5		Genetics and Inheritance (II) – DNA Replication and Gene Expression
6		Genetics and Inheritance (III) – DNA Mutations and their Consequences
7		Genetics and Inheritance (IV) – Molecular Techniques in DNA Analysis
8		Genetics and Inheritance (V) – Organization of Genome & Control of Gene Expression in Eukaryotes *[Includes Core Idea 1D: Stem Cells]*
9		Genetics and Inheritance (VI) – Organization and Inheritance of Viral Genomes
10		Genetics and Inheritance (VII) – Organization of Genome & Control of Gene Expression in Prokaryotes
11		Genetics and Inheritance (VIII) - Inheritance
12	**Core Idea 3** Energy and Equilibrium	Communication and Equilibrium in Multicellular Organisms
13	**Core Idea 4** Biological Evolution	Biological Evolution
14	**Extension Topic A** Infectious Diseases	Immunity and Infectious Diseases
15	**Extension Topic B** Impact of Climate Change on Animals & Plants	Climate Change – Causes and Impacts on Animals and Plants

TOPIC SYNOPSIS

An understanding of *Genetics and Inheritance* that would help make sense of the transition from molecular to organismal level. *Genetics and Inheritance* provides the molecular basis to the understanding of how variations in populations arise and this is important in the study of biological evolution. At the cellular level, expression of genes involves cellular structures such as the nucleus, endoplasmic reticulum and ribosome. Many essential products of gene expression are enzymes involved in biochemical pathways which control physiological functions. As such, mutation of genes may give rise to dysfunctional proteins which in turn could result in diseases. Sickle cell anemia and cancer are some examples of genetic diseases.

The following questions should help you frame your learning:

- How does the genetic make-up of an organism and the environment influence the organism's appearance, behavior and survival?
- How does the inheritance of genetic information ensure the continuity of humans as a species?

Heritable information, in the form of DNA (and in some cases RNA), provides for continuity of life

Genomes contain heritable information necessary for continuity of life at all levels: cell, organism and system. This information is stored and passed on to subsequent generations via DNA. Reproduction can occur at the cellular or organismal level; each progeny needs to receive heritable genetic information from its parent(s). The process of replication is thus vital to ensure that such genetic information is passed from the parents to the offspring.

Genetic information is stored in an organism's DNA; expression of genes results in the synthesis of functional products, such as rRNA, tRNA and proteins. These products play a role in intra- and extra-cellular biochemical pathways and influence the physiological processes in organisms.

But how does a gene, which consists of a string of DNA hidden in a cell's nucleus, know when it should express itself? How does this gene lead to the production of a string of amino acids called a protein? How do different types of cells know which types of proteins they must manufacture? The answers to such questions lie in the study of gene expression. This topic begins by showing how a quiet, well-guarded string of DNA is expressed to make RNA and how the messenger RNA is translated to form a protein. Along the way, the article set also examines the nature of the genetic code, how the elements of code were predicted, and how the actual codons were determined.

LEARNING OUTCOMES

Core Idea 2A: The Structure of Nucleic Acids and Gene Expression
The structure of DNA was proposed by Watson and Crick in 1953. With an understanding of DNA structure, experimental evidence supported the proposal that DNA replicates in a semi-conservative manner. The central dogma states that genetic information is encoded in the DNA and transferred to the mRNA during transcription. In addition to mRNA transcription, tRNA and rRNA are transcribed; tRNA is needed during translation while rRNA is a component of ribosomes. In eukaryotic transcription, pre-mRNA is synthesised and then processed to produce mature mRNA. Subsequently, through translation, the information on the mRNA is used to synthesise polypeptides, which are folded into functional proteins.

Candidates should be able to:

a) Describe the process of DNA replication and how the end replication problem arises.

b) Describe how the information on DNA is used to synthesise polypeptides in prokaryotes and eukaryotes. (Description of the processes of transcription, formation of mRNA from pre-mRNA and translation is required)

LECTURE OUTLINE

1. The Central Dogma of Molecular Biology

2. DNA Replication
- 2.1 Experimental evidence for the mechanism of DNA replication: The Meselson-Stahl experiment
- 2.2 Mechanism of semi-conservative DNA replication
- 2.3 The end-replication problem

3. Gene Expression – Transcription and Translation
- 3.1 The concept of a gene
- 3.2 The genetic code
- 3.3 Gene expression
  - 3.3.1 Transcription
  - 3.3.2 Post-transcriptional modification
  - 3.3.3 Translation
  - 3.3.4 Post-Translational modification
- 3.4 Summary: Roles of RNAs in protein synthesis

4. Comparisons
- 4.1 Transcription vs. Translation
- 4.2 DNA replication vs. Transcription
- 4.3 DNA replication vs. Translation

REFERENCES
1) Campbell 9th Edition pages 305-318, 325-343

Web-links And Animations

DNA Replication

DNA Replication process	Meselson-Stahl experiment
https://www.youtube.com/watch?v=9EwNHIMfKR8	http://www.sumanasinc.com/webcontent/animations/content/meselson.html

Transcription

Process including binding of transcription factors	Post-transcriptional modification / RNA processing	Transcription process
https://www.youtube.com/watch?v=WsofH466lqk	http://www.youtube.com/watch?v=O0wouZGmXwI	https://dnalc.cshl.edu/resources/3d/13-transcription-advanced.html

Translation:

Transcription, RNA splicing, and translation	Translation process	Translation (prok) showing ribosome sites
https://dnalc.cshl.edu/view/16933-3D-Animation-of-DNA-to-RNA-to-Protein.html	https://vcell.science/project/translation	https://www.youtube.com/watch?v=Ikq9AcBcohA

Pre-lecture Exercise:

How much do you remember about <u>Deoxyribonucleic Acid (DNA)</u>?

1. Nucleic acids are made up of _____ (monomers).
2. A nucleotide is made up of a _____, a _____ and a _____.
3. DNA nucleotides are called _____ while RNA nucleotides are called _____.
4. Many free nucleotides join together to form a _____ chain via condensation reactions which occur between the **<u>5' _____ group</u>** of one free nucleotide and the _____ **<u>group</u>** of the **<u>3' carbon</u>** of the previous nucleotide**, forming a _____ bond.**
5. A DNA double helix structure consists of 2 _____.
6. The two polynucleotide chains are _____, i.e. their 5' to 3' orientations run in opposite directions.
7. _____ occurs between purine and pyrimidine bases in DNA.
    - Purine bases: _____ and _____
    - Pryimidine bases: _____ and _____
    - Adenine complementary base pairs with _____
    - Cytosine complementary base pairs with _____
8. Stability and integrity of the double helix is maintained by _____ **bonds** between the complementary bases and _____ **interactions** between the stacked bases.

1. The Central Dogma of Molecular Biology

- When Watson and Crick suggested that DNA exists in the form of a double helix, they also suggested that the genetic information, which controls the activities of the cell, might be passed from generation to generation.

- This **genetic information** is **stored** in the form of the **sequence of nucleotides** in **DNA molecule.**

- The **sequence of nucleotides in the DNA** is a **code for the sequence of nucleotides in RNA** and **subsequently,** the **sequence of amino acids in protein molecules.**

- This **flow of genetic information** has since been known as the **central dogma of molecular biology** (a term coined by Crick).

- The Central Dogma refers to (Refer to Fig. 1.1):

    - **The genetic information** that is **stored as DNA** in all organisms (including viruses).

    - **Replication** of the genetic information **involves DNA-directed DNA synthesis** using DNA as a template to synthesize DNA.

    - When the **genetic information** is **expressed in a cell**, it flows ***uni-directionally***
        - from **DNA to RNA** (by a process called **transcription**)
        - and then from **mRNA to protein** (by a process called **translation**)

- However, there are cases when the Central Dogma **<u>does not</u>** hold true:

    - In cases when a gene codes for a type of RNA and the flow of information stops at RNA (e.g. rRNA, tRNA, etc).

    - A reverse flow of information from RNA to DNA (by a process called reverse transcription) (Refer to Topic 9: Organization & Inheritance of Viral Genomes).

    - RNA, instead of DNA is the form of the genetic information of the organism (e.g. some viruses).

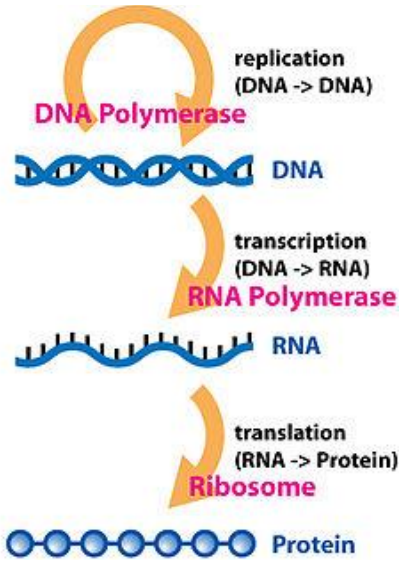


Fig. 1.1. The Central Dogma of Molecular Biology

2. DNA Replication

In 1953, James Watson and Francis Crick shook the scientific world with an elegant double-helical model for the structure of DNA. Over the past 60 years, their model has evolved from a novel proposition to an icon of modern biology. DNA, the substance of inheritance, is the most celebrated molecule of our time.

Of all nature's molecules, nucleic acids are unique in their ability to direct their own replication from monomers. Hereditary information is encoded in the chemical language of DNA and reproduced in all cells in your body. It is this DNA programme that directs the development of your biochemical, anatomical, physiological and, to some extent, behavioral traits.

***Excerpts from Campbell, N.A. and Reece (2011) Biology. 9th Edition. The Benjamin/Cummings Publishing Company, Inc. - Chapter 16, The Molecular Basis of Inheritance.*

Key roles of DNA:

- **Long-term storage of genetic information** for development and function of organisms.
- **Each strand acts as a template** to make more copies of DNA in **DNA replication**.
- Acts as a **template for transcription** (Section 3).

Most important **structural features of DNA** to note for DNA replication:

- **Double stranded**
- **Antiparallel** (one strand runs from 5' to 3' direction, the other strand runs from 3' to 5' direction)
- **Complementary base pairing** between Adenine with Thymine, Cytosine with Guanine

Some key questions:

(a) What is DNA replication?

- The ***process*** of **doubling the amount DNA in a cell / making copies of DNA** is known as DNA replication.

(b) When does DNA replication occur?

- For **eukaryotes** - Occurs during the **S-phase of interphase** during the cell cycle, when a cell prepares for mitosis or meiosis.
- For **prokaryotes** – Occurs at the start of binary fission (Topic 10).

(c) Where does DNA replication occur?

- Nucleus, mitochondria and chloroplasts of eukaryotes
- Cytoplasm of prokaryotes

(d) Why does DNA replication occur?

- In mitosis - Nucleus **divides once**. DNA replication doubles the amount of DNA in the nucleus such that when chromatids separate into the **two resulting daughter cells**, each will have exactly the **same amount** of DNA as the parental cell (**to ensure genetic stability**).

- In meiosis – Nucleus **divides twice**. DNA replication doubles the amount of DNA so that meiosis results in **four daughter cells**, each having **half the amount** of DNA compared with the parental cell. (Reduction in ploidy for fertilization)

(e) How does DNA replication occur? (Section 2.2)

2.1 Experimental evidence for the mechanism of DNA replication:

The Meselson-Stahl experiment

- Before the mechanism of DNA replication was known, there were **three <u>proposed</u> models** (Fig. 2.1a).

(a) Semi-conservative Model

- The **two strands** of the DNA molecule **separate** by breaking the hydrogen bonds between base pairs of the two strands.
- **Each strand** then acts as a **template** for the formation of one new strand.
- **New hydrogen bonds** form between bases of one old and one new strand to form a complete molecule.
- Each daughter cell **inherits a resulting DNA molecule** that consists of **one new** and **one old strand**.

(b) Conservative Model

- The **entire double-stranded DNA molecule** acts as a **template** for an entirely new DNA molecule to be formed.
- The **parent DNA molecule is intact** and goes into one daughter cell. The new molecule goes into the other daughter cell.

(c) Dispersive Model

- Parental DNA breaks up into **short segments**.
- These segments are used as **templates** for the synthesis of a new double helix.
- The segments are then somehow **joined together**.

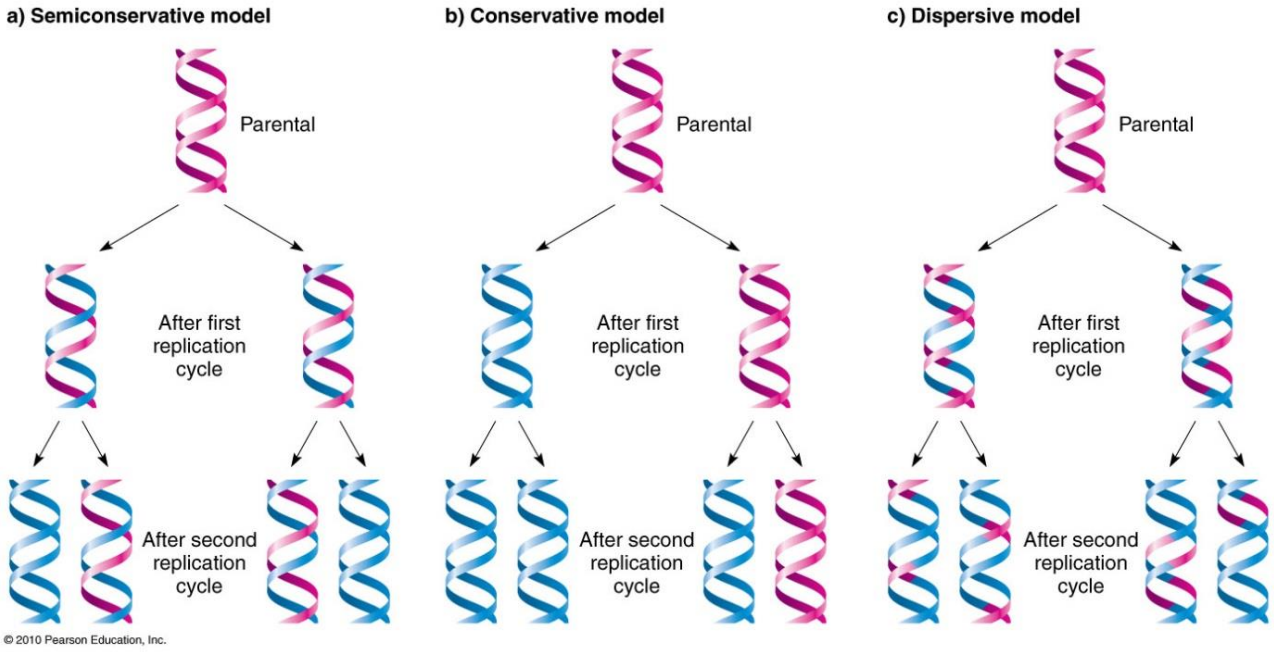


Fig. 2.1a: Possible models of DNA replication.
(a) Semi-conservative model (b) Conservative model (c) Dispersive model

- The three models were tested by Mathew Meselson and Franklin Stahl in 1958 in an experiment now known as the **Meselson-Stahl Experiment**.

- Materials used:
  - *E. coli* bacteria culture
  - Culture medium containing ^{15}N (**heavy** isotope)
  - Culture medium containing ^{14}N (**lighter** isotope)
  - Kit to extract bacterial DNA from all culture medium
  - Centrifuge for separation of bacterial DNA in band(s) according to their densities in a solution of caesium chloride (a very dense solution)

- Experimental details: (Fig 2.1b)

  1. Parental *E. coli* was grown in ^{15}N medium as the only source of nitrogen for **many generations** until **^{15}N** was incorporated into the **nucleotide bases** in **all bacterial DNA**.

  2. The *E. coli* containing ^{15}N was then **transferred into a medium containing only ^{14}N**.
  - To distinguish between 'old' and 'new' DNA, two different isotopes of nitrogen were used: ^{14}N (more common isotope) and ^{15}N (less common, **heavier** isotope).

  3. The transferred *E. coli* was then allowed to **divide for <u>one generation</u>** and some cells were then collected.

  - These collected cells were called the '**first generation cells**'.

  - **DNA was then extracted** from these cells, and the relative mass determined by **ultracentrifugation** (60000 revolutions per min) in **caesium chloride solution**. (Fig 2.1c)

  4. The **remaining cells** were allowed to **divide once more** to obtain **'<u>second generation cells</u>'**. DNA was extracted again and centrifuged.

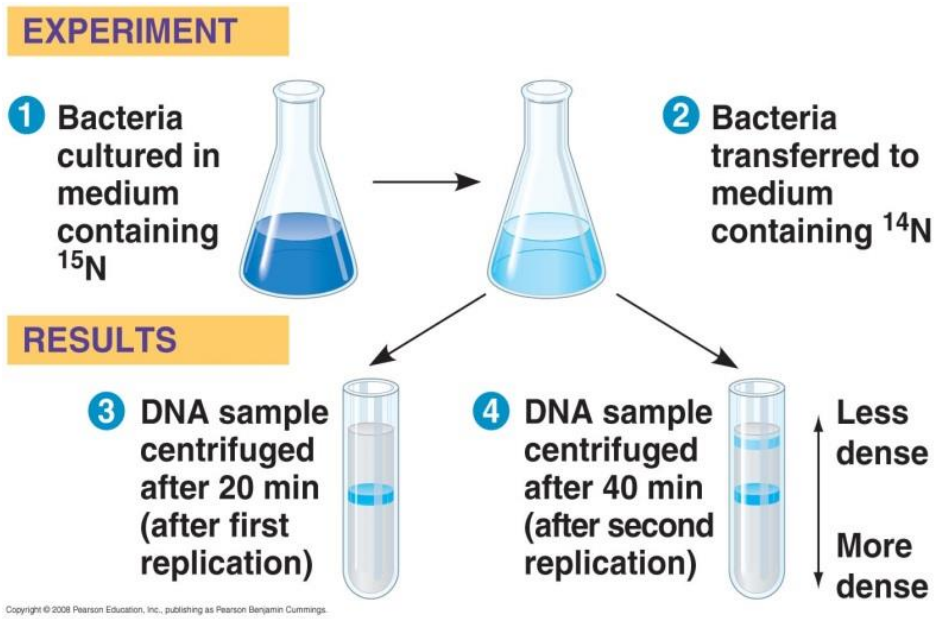


Fig 2.1b: Meselson-Stahl Experiment

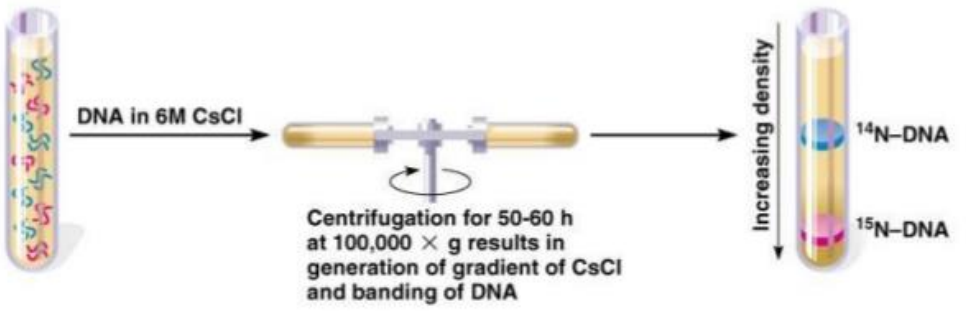


Fig 2.1c: Density gradient centrifugation in caesium chloride (Cs Cl) solution to separate DNA molecules or strands according to density

Note:

- Centrifugation separates DNA molecules of **different densities**.
- Double helix DNA molecule containing only **$^{15}N^{15}N$** is **denser** than double helix DNA molecules containing only **$^{14}N^{14}N$**.
- Thus, **$^{15}N^{15}N$** -DNA would form a band below that of the **$^{14}N^{14}N$** -DNA in the caesium chloride solution.
- **Hybrid** DNA **^{14}N ^{15}N** which is made up of one **^{14}N** strand and one **^{15}N** strand would form a band between the **$^{14}N^{14}N$** and **$^{15}N^{15}N$** bands.

Observations (Fig. 2.1d) and explanation of results that illustrate semi-conservative DNA replication.

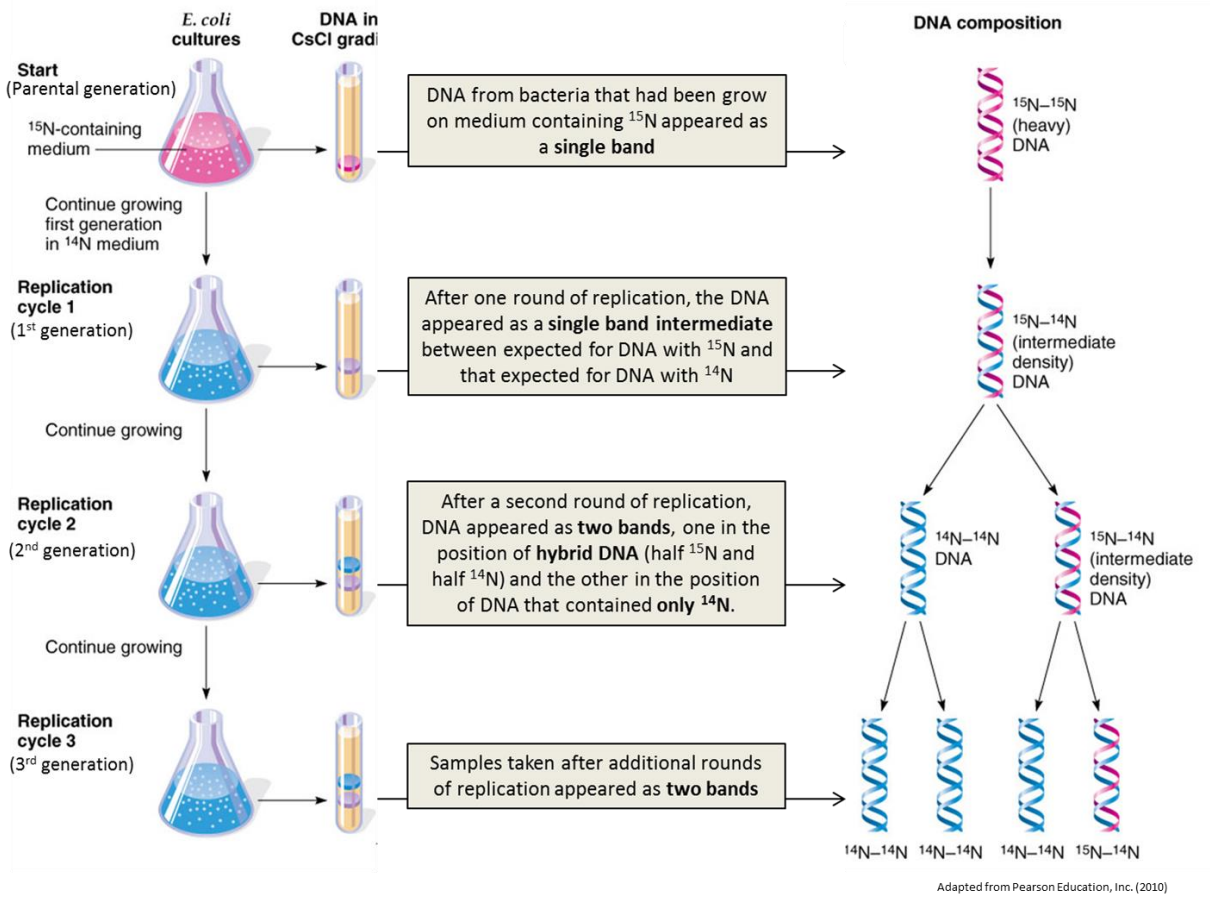
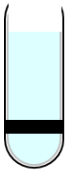
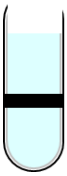
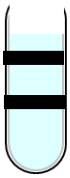


Fig. 2.1d: Experimental procedure to prove that DNA replication is semi-conservative.

	DNA from parental cells	DNA from first generation cells	DNA from second generation cells
Results	 $^{15}N^{15}N$(100%)	 $^{14}N^{15}N$ (100%)	 $^{14}N^{14}N$ (50%) $^{14}N^{15}N$ (50%)
Description and Explanation	• DNA molecules from parental cells appear as **one DNA band** with **density at ^{15}N.** • This means **all DNA molecules comprise two ^{15}N DNA strands.** • As they were grown in ^{15}N medium, ^{15}N is incorporated into both strands of DNA molecule.	• In the **first generation**, **one DNA band** with **density halfway between ^{15}N and ^{14}N** appears • This is because **each** of the original **^{15}N DNA strand** from parental cells **acts as a template for the formation of a new ^{14}N strand** in the first generation cells. • Hence, **all DNA molecules** in the first generation cells comprise **one original ^{15}N DNA strand** and **one new ^{14}N DNA strand.**	• In the **second generation**, **two DNA bands** were seen. One DNA band appears with **density at ^{14}N** and the other DNA band appears with **density halfway between ^{15}N and ^{14}N.** • This is because each strand of **$^{15}N^{14}N$ DNA molecule** in the first generation cells **acts as a template for the formation of the new ^{14}N strand** in the second generation cells. • Hence, **50%** of the resulting molecules comprise **one ^{15}N DNA strand** and **one new ^{14}N DNA strand**, while the **other 50%** of the resulting molecules comprise **two ^{14}N DNA strands**.

2.2 Mechanism of semi-conservative DNA replication

- DNA replication occurs during the **S phase of interphase** of the cell cycle (Fig. 2.2a), in the **nucleus** of eukaryotic cells.

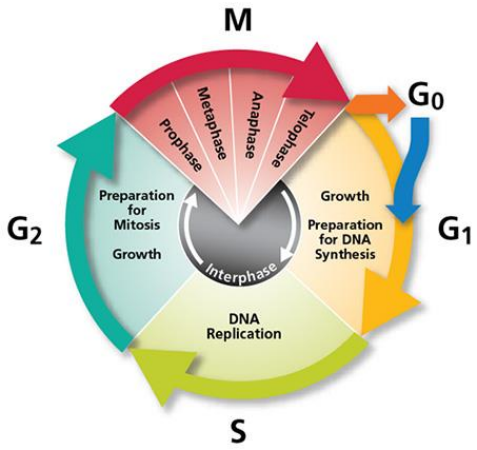


Fig. 2.2a: DNA replication occurs in the S phase of the cell cycle.
The preceding G_1 phase is a time for the cell to prepare for DNA replication.

- However, because prokaryotic cells do not contain a nucleus, this process occurs in the **nucleoid region** in the cytoplasm.

- Mechanism of DNA replication is largely similar in eukaryotes and prokaryotes. DNA replication will be illustrated here using the prokaryotic model.

- Before DNA replication occurs, **molecules required** for DNA replication are **manufactured in the cytoplasm** and **imported into the nucleus** via the nuclear pores.

    - These molecules include: **deoxyribonucleoside triphosphates (deoxyribonucleotides)**, **ATP** and various ***subunits*** **of enzymes and proteins**.

    - The enzymes and proteins required for DNA replication are summarized below:

Proteins involved	**Function**
DNA helicase	Unwinds and unzips the DNA double helix by breaking the hydrogen bonds between complementary bases.
Single-stranded DNA binding proteins	Prevent reannealing of the double strands by stabilising the single-stranded DNA formed by the action of helicase.
Primase	Synthesises RNA primer which provides a free 3'OH group for DNA polymerase III to add new deoxyribonucleotides.
DNA polymerase III	• Elongates DNA by adding deoxyribonucleotides to the free 3' OH group of the primer by complementary base pairing. • Catalyses the formation of phosphodiester bonds between adjacent deoxyribonucleotides.
DNA polymerase I	• Proofreads the newly synthesized strand. • Replaces RNA primer with deoxyribonucleotides via complementary base pairing and formation of phosphodiester bonds.
DNA ligase	Seals the gaps between Okazaki fragments by forming phosphodiester bonds between the Okazaki fragments.

1. Start of DNA replication

- DNA replication is **bi-directional**, meaning that the synthesis of each **new DNA strand** occurs in opposite directions.

- The process **begins at** particular sites called **origins of replication *(ori)***, which are **short stretches of DNA with specific sequence** of nucleotides.

- **Eukaryotic DNA** has **many** origins of replication on **each DNA molecule** (Fig. 2.2b).

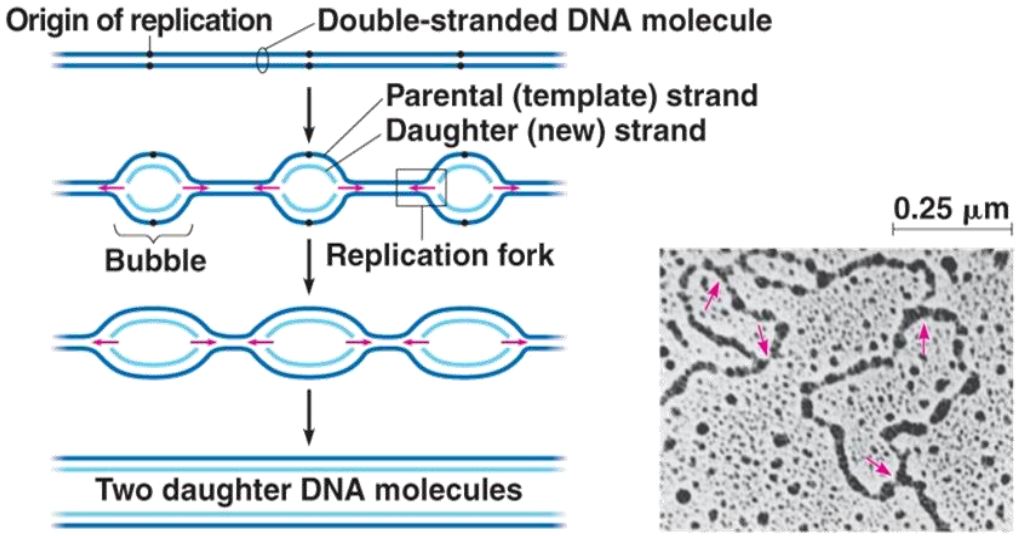


Fig. 2.2b: Multiple origins of replication in Eukaryotic DNA

- Unlike eukaryotic DNA, **prokaryotic DNA** has only **one origin of replication** on its circular DNA (Fig. 2.2c).

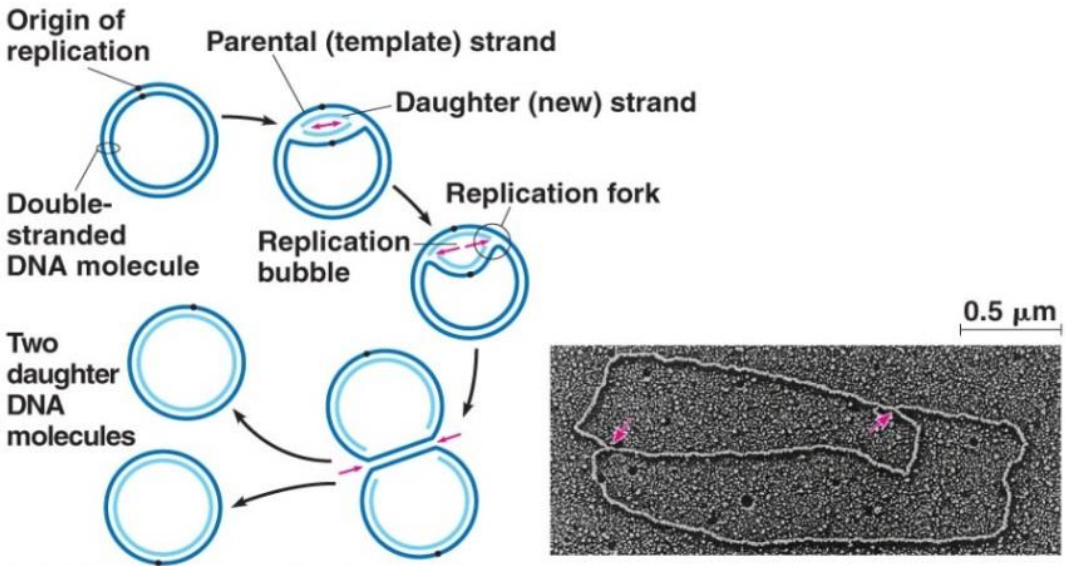


Fig. 2.2c: One origin of replication in the *E.coli* chromosome

Why do eukaryotes require many origins of replication in each chromosome?

- Various proteins (including enzymes) first bind near the origin of replication to form a protein complex, which will initiate DNA replication (Fig. 2.2d).

(i) DNA Helicase

- DNA Helicase is an enzyme that **<u>unwinds and unzips</u>** the double stranded DNA **into single strands,** allowing each strand to be copied.

- **Unzipping of DNA** involves **breaking the hydrogen bonds between base pairs** in **double stranded DNA.** This **requires energy** in the form of **ATP**.

- The ***initial*** **unzipping** of the DNA molecule leads to the **formation of** a **replication bubble**. **At each end** of the replication bubble is a **replication fork**, where the unzipping of the DNA molecule will continue. Hence, there are 2 replication forks for each replication bubble.

- Each single DNA strand is known as a **parental strand** and acts as a **template** for the synthesis of a new complementary daughter strand.

(ii) Single-stranded Binding Proteins (SSBPs)

- After separation, **single-stranded binding proteins bind** to the **unzipped parental strands** and **prevent** them from **reannealing,** until new deoxyribonucleotides form complementary base pairs with the parental strand via hydrogen bonds.

(iii) Topoisomerase

- The separation of the double helix causes tighter strain <u>ahead</u> of the replication fork. Topoisomerase helps to relieve this strain by breaking, swivelling and re-joining each DNA strand.

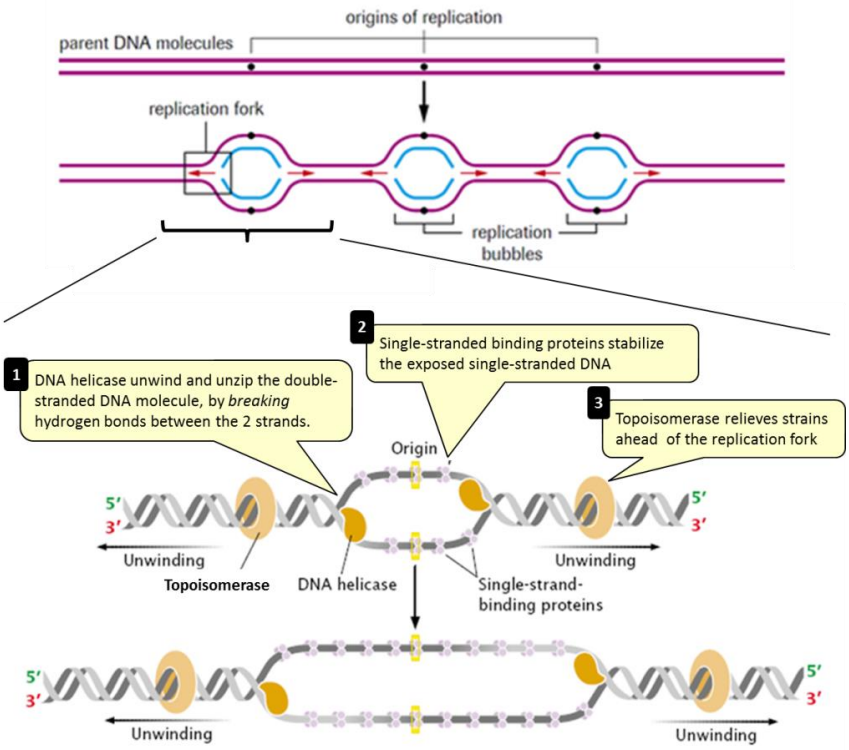


Fig. 2.2d: Shows the proteins involved in the initiation of DNA replication. Note that there are two replication forks proceeding in opposite direction.

2. Priming of DNA by Primase

- **Before** the **elongation** of the new daughter DNA strand can proceed by DNA polymerase III, the enzyme **primase** is needed to **initiate** the elongation process (Fig. 2.2e).

(iv) Primase

- Primase **catalyses** the **addition of a short sequence of RNA primer** (note: not DNA) to the start of the new strand.
- Primers are **short sequences** (5 to 10 nucleotides long) of **RNA** that are **complementary to** the **DNA parental strands (template)**.
- Primers **provide** the **free 3' OH group for DNA polymerase III to add new deoxyribonucleotides**.
- The **RNA primers are later replaced with deoxyribonucleotides** by another DNA polymerase, called DNA polymerase I.

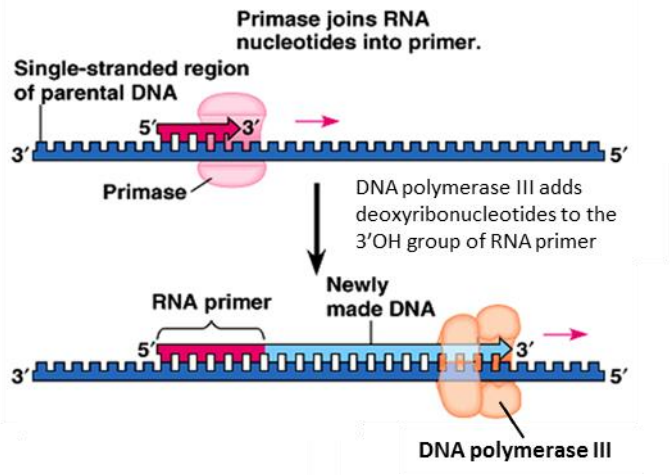


Fig. 2.2e: Priming of DNA replication and removal of RNA primers.

Why is the addition of RNA primer necessary? Why can't DNA polymerase III initiate DNA synthesis directly?

3. DNA elongation

- DNA elongation is carried out by **DNA polymerase III**.

(v) DNA polymerase III

- DNA polymerase III catalyses the elongation of the new strand by **adding deoxyribonucleotides** to a **free 3'OH group** (Fig. 2.2f).
  - It can only add deoxyribonucleotides to an existing 3'OH group. Hence the addition of a primer, which provides the first free 3'OH group, is essential.
- DNA polymerase III recognizes the bases on the parental strand and selects complementary, **free deoxyribonucleotides** to add to the growing end of the new strand by **complementary base pairing**. These complementary bases form **hydrogen bonds** with each other.
- DNA polymerase III synthesizes the growing **daughter strand** in the **5' to 3' direction** by **catalysing the formation of phosphodiester bonds between adjacent deoxyribonucleotides** of the daughter strand.
- While doing so, it **reads** the **parental strand** in the **3' to 5' direction**.

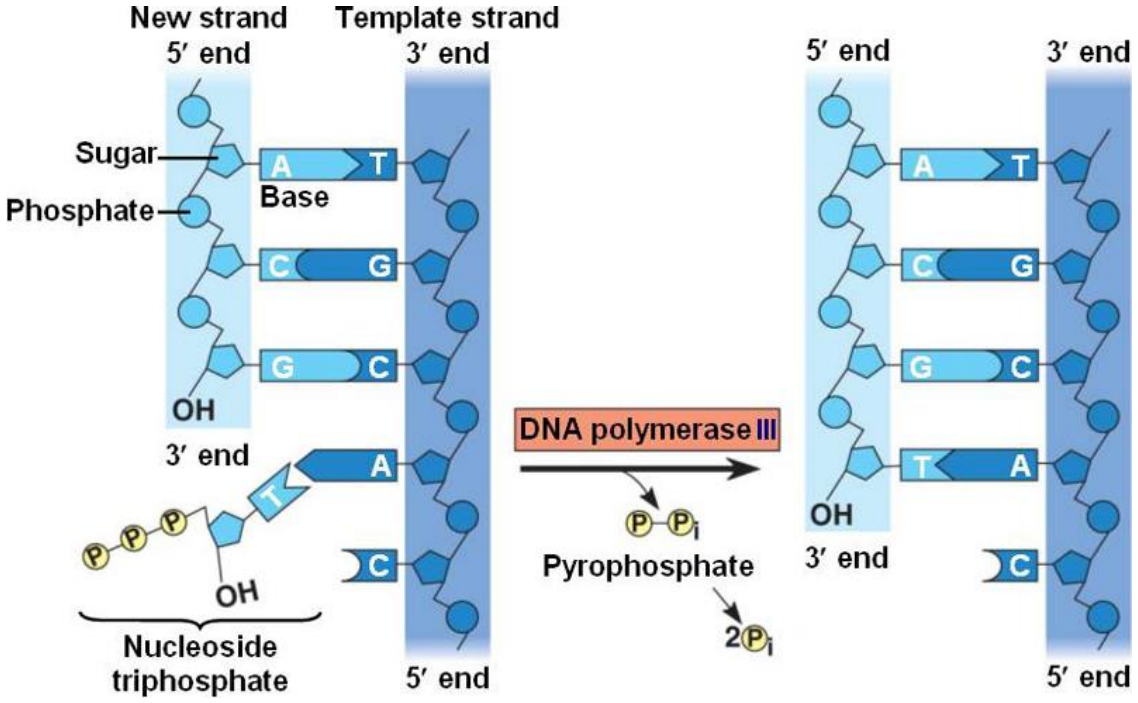


Fig. 2.2f: Addition of deoxyribonucleotides to newly synthesized daughter strand occurs at the 3' end of the new daughter strand

- As DNA polymerase III elongates the daughter strand, it proofreads the deoxyribonucleotide added to ensure that the correct one was added. When an **incorrect deoxyribonucleotide** is added, the **DNA polymerase III** will excise and **replace it with the correct deoxyribonucleotide** (Fig. 2.2g).

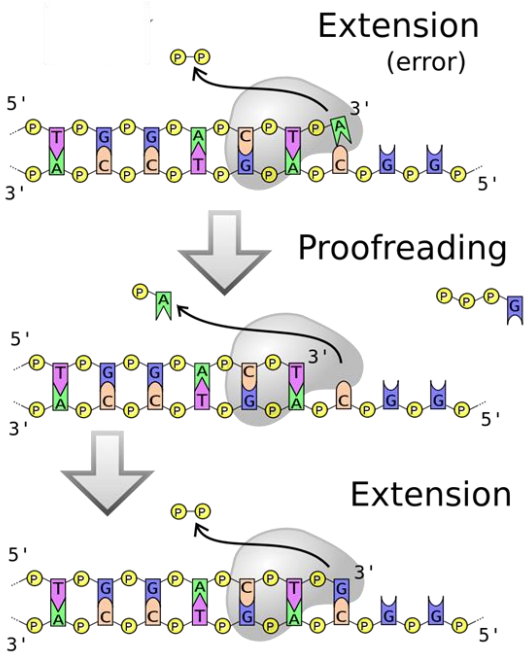


Fig. 2.2g: Elongation by DNA polymerase III

- As the parental DNA strands run in **<u>antiparallel</u>** direction, elongation proceeds in **<u>two directions</u>** – towards and away from replication fork (Fig. 2.2h).

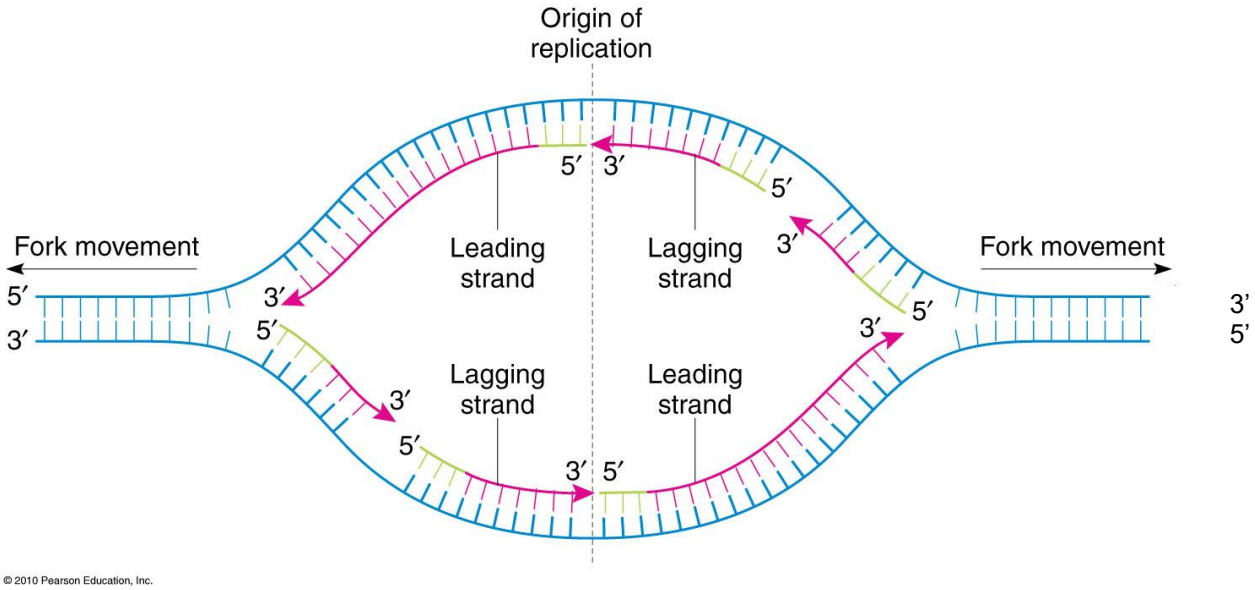


Fig. 2.2h shows an overview of how DNA replication occurs bi-directionally.
Note that elongation of new DNA strands occur in the <u>5’ to 3’ direction for both leading and lagging strands</u>.

Direction 1: Synthesis of the **leading strand** (Fig. 2.2i):

- is **continuous in a 5' to 3'** direction of the new daughter strand.
- DNA Polymerase III moves **towards** the replication fork.
- **only one primer** is needed.

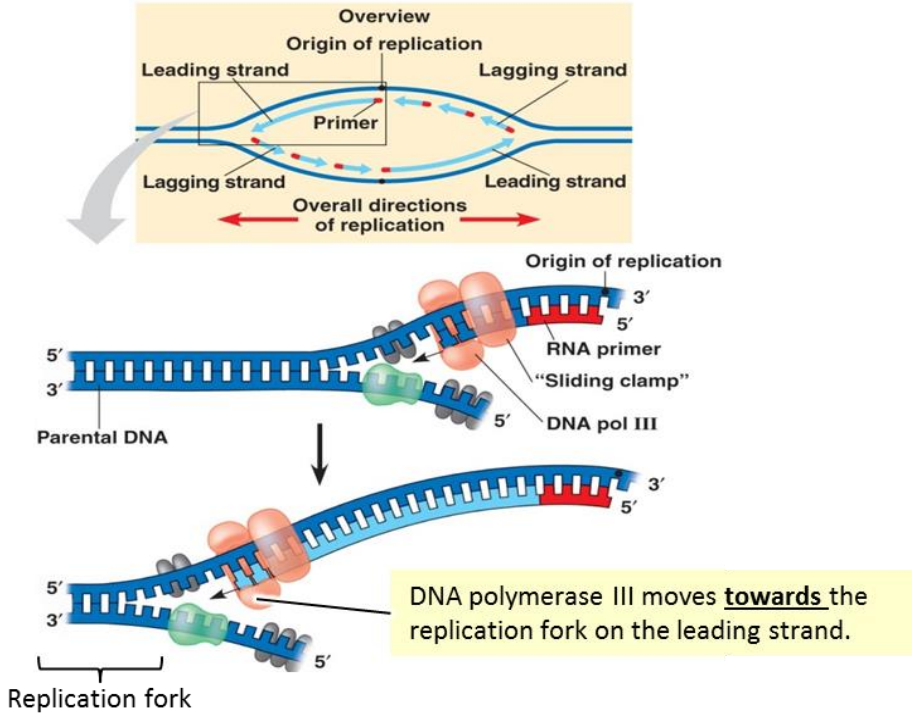


Fig. 2.2i: DNA elongation of leading strand.

Direction 2: Synthesis of the **lagging strand** (Fig. 2.2j):

- is **discontinuous**, but also in the **5' to 3' direction**.
- creates fragments called **Okazaki fragments**.
- DNA Polymerase III moves **away** from the replication fork.
- **Many primers** are synthesized, as one primer is needed for every Okazaki fragment.

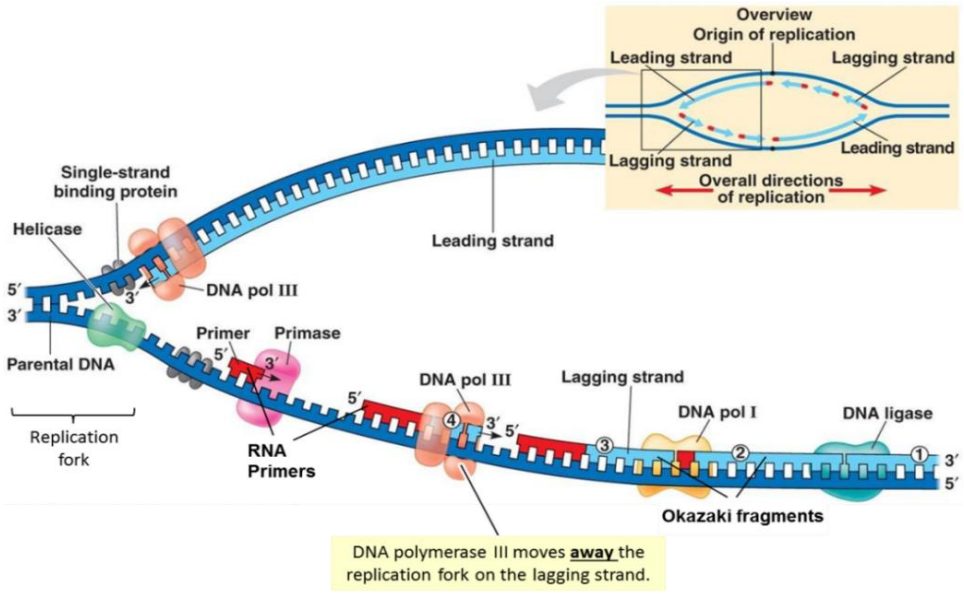


Fig. 2.2j: DNA elongation of lagging strand

4. DNA proofreading, replacement of RNA primers and ligation

- **Proofreading** and **replacement of RNA primers by DNA polymerase I**

(vi) DNA polymerase I

- As elongation proceeds, another DNA polymerase, **DNA polymerase I**, **proofreads** the synthesized region on the daughter strand by checking for correct base pairings.
- **Deoxyribonucleotides** that are **incorrectly paired** are **replaced**.
- **RNA primers** are later **excised** and **replaced with deoxyribonucleotides** by DNA polymerase I (Fig. 2.2j).

- **Ligation of Okazaki Fragments by DNA ligase**

(vii) DNA ligase

- After replacement of RNA primers with deoxyribonucleotides, the **gaps between** the **Okazaki Fragments** are **ligated** (joined) by the enzyme, **DNA ligase,** via the **formation of phosphodiester bonds** (Fig. 2.2k).

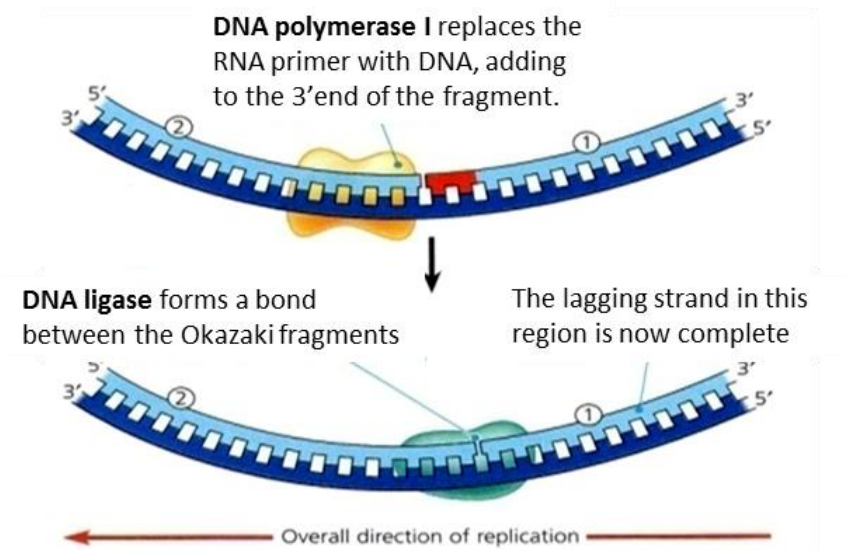


Fig. 2.2k: Replacing RNA primers with deoxyribonucleotides by DNA polymerase I and ligation of Okazaki fragments by DNA ligase

5. <u>End of DNA replication (products of DNA replication)</u>

- As replication proceeds, the **complementary parental and daughter strands rewind progressively** into a **new double helix** (Fig. 2.2l).

- The process is **semi-conservative** since **each resulting DNA molecule (double helix) consists of one old parental strand** and **one new daughter strand** (Fig. 2.2l).

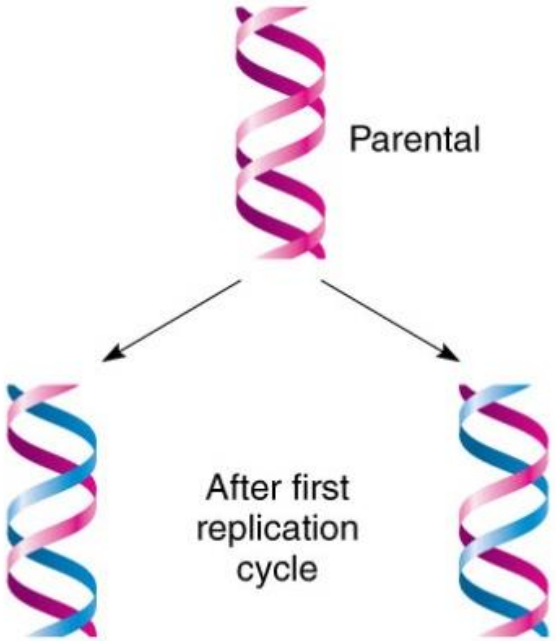


Fig 2.2l: After the process of semi-conservative DNA replication, the resultant DNA molecule consists of one old (parental) strand and one new (daughter) strand.

Summary of DNA replication

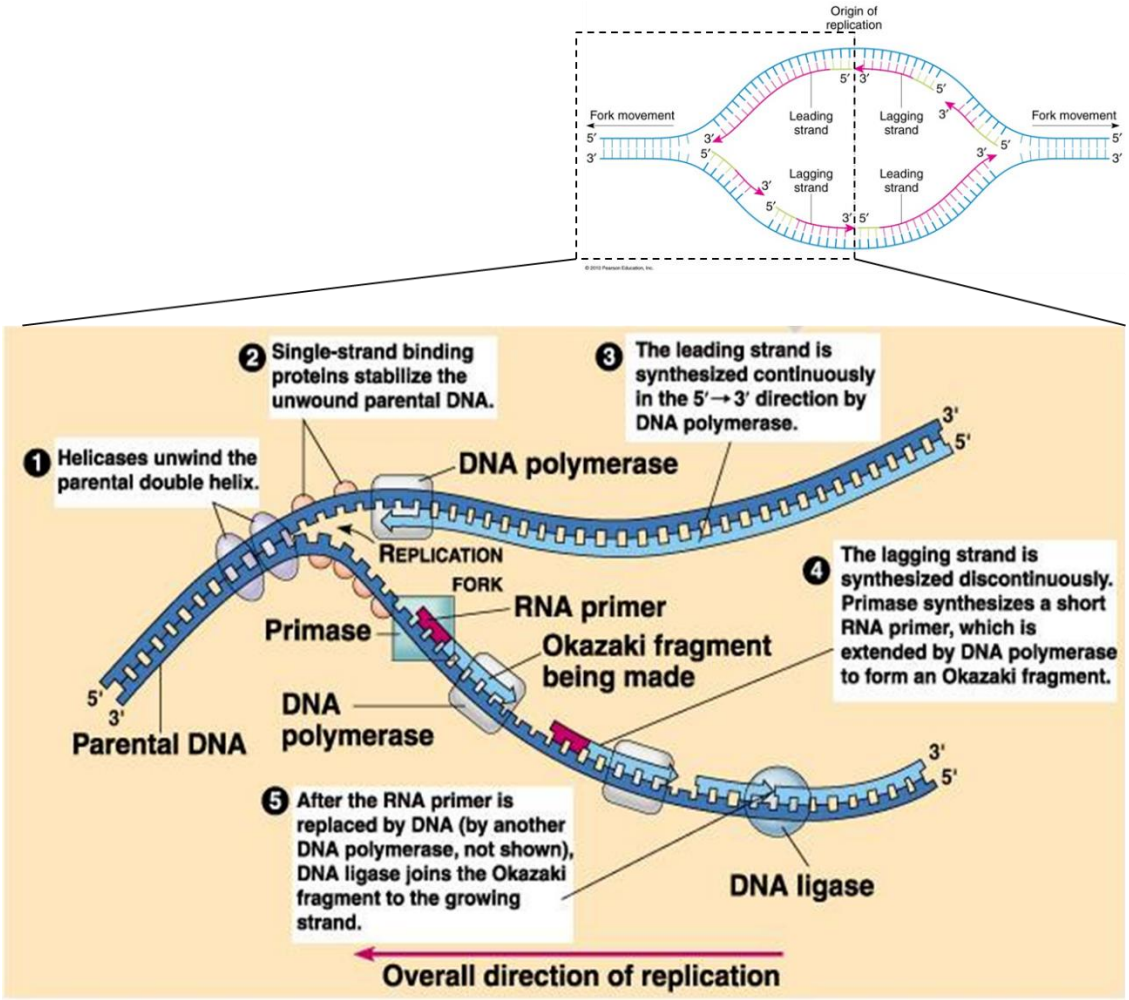


Fig. 2.2m: Summary of DNA replication and the components involved at <u>one</u> replication fork.
Note that each Okazaki fragment on the lagging strand is synthesized away from the replication fork, but the overall direction of synthesis of both strands is towards the replication fork.

2.3 The end-replication problem in Eukaryotic cells

- The ends of linear chromosomes pose a problem for the usual DNA replication machinery.
- For the leading strand, synthesis can continue to the end of its template. However, for the lagging strand, replacing the final RNA primer with DNA at the **5' end** of the daughter DNA strand would **not** be possible. This is because DNA polymerase can only add deoxyribonucleotides to the free 3'-OH end of a pre-existing polynucleotide but not to the 5' end.
- As a result, a dividing cell that undergoes repeated rounds of replication will produce shorter and shorter DNA molecules (Fig. 2.3a).

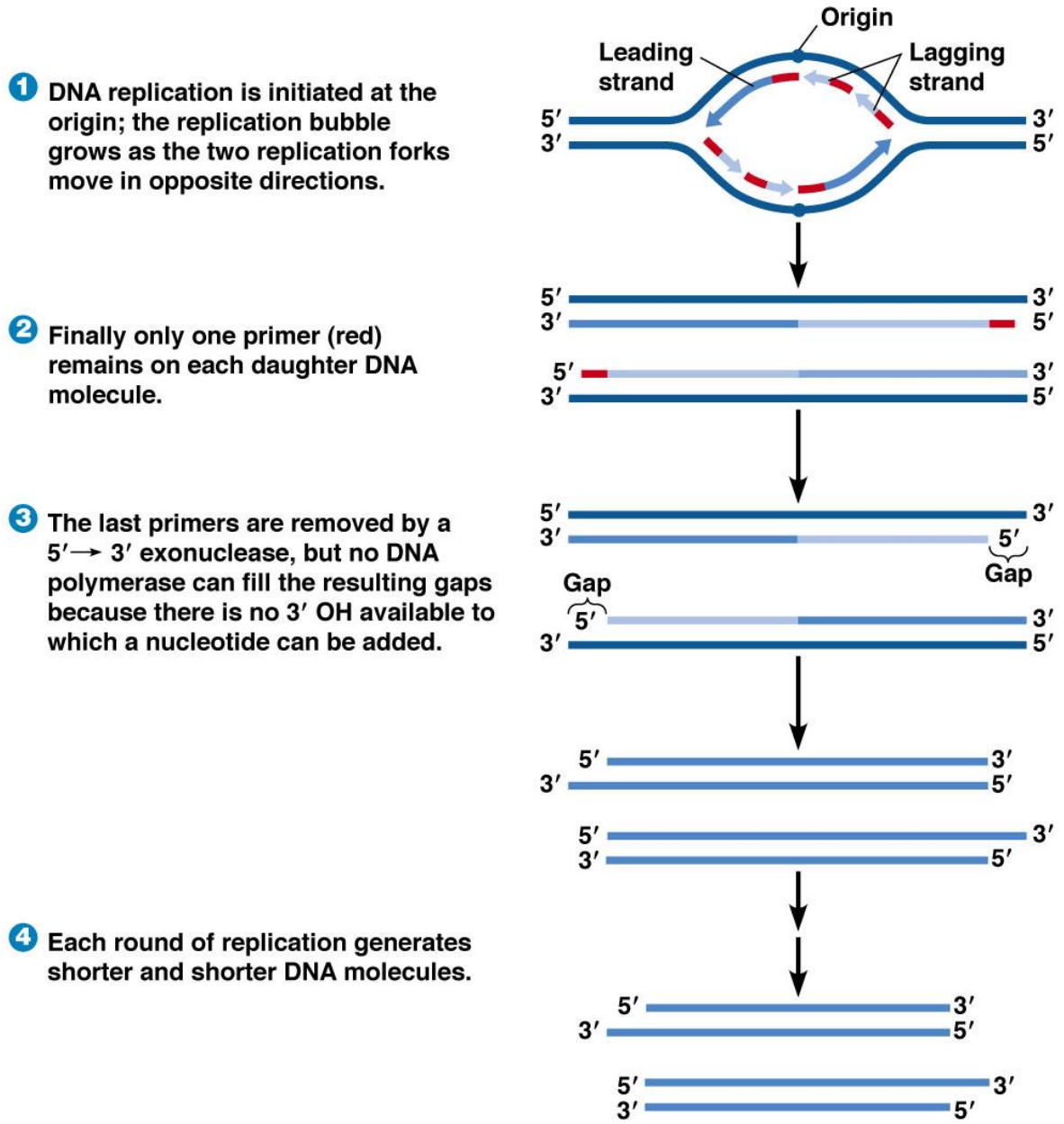


Fig. 2.3a: The end replication problem results in shorter molecules of DNA after each round of DNA replication.

- The ends of a linear DNA are known as **telomeres**.
    - Telomeres are **short**, **repeating**, **non-coding** DNA sequence found at the **two ends of the DNA / chromosome**.
    - The exact sequence of the repeats in a telomere depends on the species. The telomeres of **humans** consist of a repeat of six nucleotide sequences, **5' TTAGGG 3'** (Fig 2.3b).
    - Since telomeres are non-coding, they help to **buffer against the loss of essential genes** after each round of DNA replication.

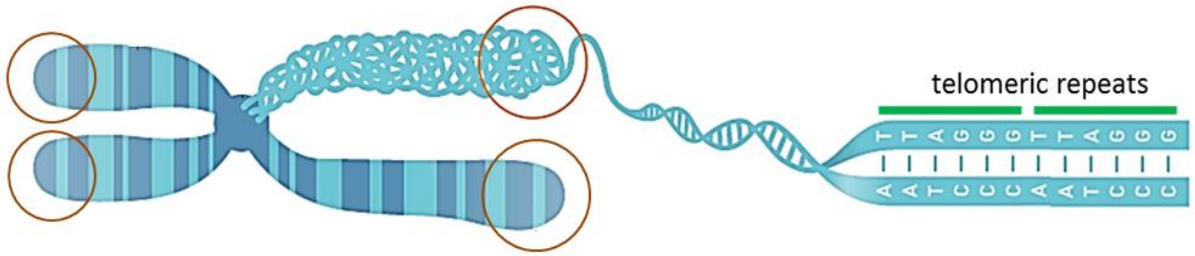


Fig. 2.3b: Telomeric sequence is found at the ends of a DNA molecule.

Do prokaryotes face the same end replication problem? Why?

- When the telomere length reaches a critical limit, the cell stops dividing and/or undergoes apoptosis (programmed cell death).

3. Gene Expression – Transcription and Translation

- Most important **structural features of RNA** to note for transcription:
  - RNA is usually **single stranded**.
  - Adenine complementary base pairs with **Uracil**, while Cytosine complementary base pairs with Guanine.
  - There are 3 main types of RNA, each with different functions;

(1) Messenger RNA (mRNA)
- **Conveys genetic information from nucleus to ribosome** as DNA is too large to leave the nucleus.
- **Acts as a template** for ribosome to carry out **translation.**
- Each mRNA molecule has a sequence of ribonucleotides. **Each triplet of bases on the mRNA is known as a codon. Each codon codes for one amino acid.**

(2) Transfer RNA (tRNA)
- **Carries specific amino acids to mRNA on ribosome** for protein synthesis
- Has an **anti-codon** to complementary base pair with a codon on mRNA

(3) Ribosomal RNA (rRNA)
- Associates with ribosomal proteins to form ribosome for translation.
- During translation, rRNA acts as a ribozyme providing peptidyl transferase activity, catalyzing the formation of peptide bonds between the amino acids.
  (More details on roles of rRNA → Refer to Topic 2)

3.1 The concept of a gene

- Each type of living organism must have distinct genetic messages that define various aspects of that organism.

- The sequence of nucleotides in the DNA molecule is used as a code for this purpose. This code determines which amino acids are joined together to form polypeptides.

- The DNA molecule is a very large molecule. Along this molecule, there are many distinct segments of deoxyribonucleotides each coding for a specific protein (Refer to Fig. 3.1). Each distinct segment of deoxyribonucleotides which codes for a protein is called a gene.

- Definition of a gene: A **gene** is a **discrete unit of hereditary information** consisting of **specific nucleotide sequence in DNA** (or RNA in some viruses).

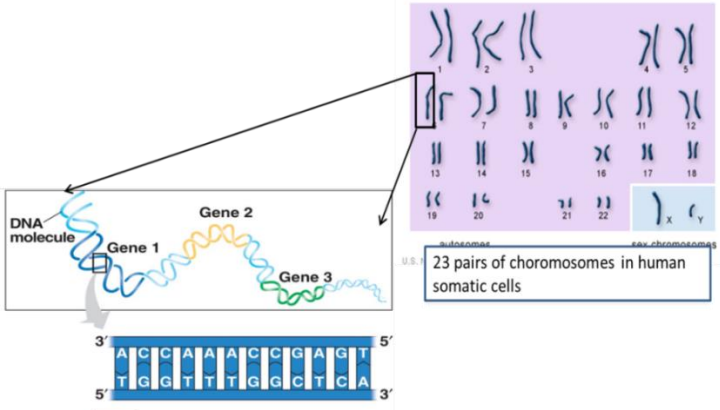


Fig. 3.1: There are many genes along a DNA molecule, each gene having a unique sequence of deoxyribonucleotides. Each chromosome under the microscope is one DNA molecule.

3.2 The genetic code

- Based on the Central Dogma, the genetic code is stored in the **sequence of deoxyribonucleotides in DNA**, which is transcribed into a specific sequence of nucleotides in mRNA during the process of transcription. The mRNA is then translated into an amino acid sequence (Fig. 3.2a).

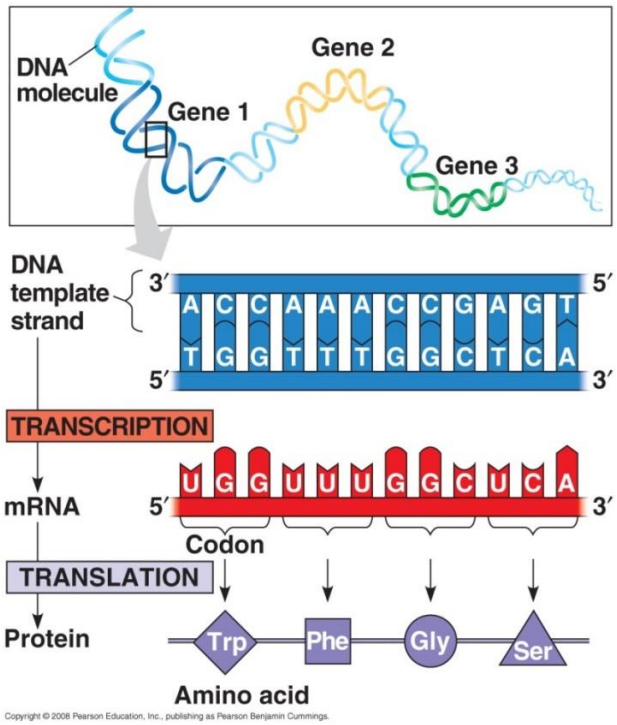


Fig. 3.2a: The flow of genetic information from DNA to protein (the Central Dogma)

- In nature, there are only **4 types of nucleotides** in DNA and RNA respectively. However, there are a total of **20 types of amino acids** that can be found in proteins.

- Thus, the **number of bases/nucleotides required to code for each of the 20 amino acids** can be found by the following formula: $\mathbf{N^c}$

  where by N = Types of Bases/Nucleotides Available (i.e. A, T, C, G, etc.)
  c = length of code (i.e. singlet, duplet, triplet, etc.)

  - If 1 nucleotide codes for 1 amino acid in the primary structure of a protein (a singlet code, i.e. 1 nucleotide long), the polypeptide can contain only $4^1 = 4$ types of amino acids. This is insufficient.

  - If 2 nucleotides code for 1 amino acid in the primary structure of a protein (a duplet code, i.e. 2 nucleotides long), the polypeptide can contain $4^2 = 4 \times 4 = 16$ types of amino acids. This is still insufficient.

  - If 3 nucleotides code for 1 amino acid in the primary structure of a protein (a **triplet** code, i.e. 3 nucleotides long), the polypeptide can contain $4^3 = 4 \times 4 \times 4 = 64$ types of amino acids. This is more than sufficient to code for 20 different amino acids. (Refer to Fig. 3.2b)

  - Hence, a **sequence of <u>three</u> nucleotides** (triplets) is the smallest unit that can sufficiently **code for all** (20) **amino acids**.

- **Features of the genetic code** (Fig. 3.2b)

1. The genetic code is a **triplet code**. **Each triplet** is **transcribed** into **one mRNA codon.**

    - The **DNA triplet code** is first transcribed into mRNA before a protein is made.
    - **mRNA codons** (3 nucleotides per codon) are **complementary** to that of the **DNA triplets** on the template strand.
    - Each amino acid to be incorporated into the polypeptide chain is determined by the sequence of an mRNA codon, which is in turn coded for by a sequence of a DNA triplet.

2. The genetic code is **degenerate.**

    - **More than one mRNA codon** can code for the **same** amino acid.
    - For example, glycine (Gly) can be coded for by 4 mRNA codons : GGG, GGA, GGC and GGU.
    - The **first two** of the three **nucleotides** in the **codon** are usually the same.

3. The genetic code is **punctuated.**

    - There are **start and stop codons** for the start and end of **translation** respectively.
    - Presence of a **start codon AUG** (which codes for amino acid methionine) signals the start of the translation of mRNA into a sequence amino acids.
    - The **stop codons UAA, UGA, UAG** act as 'stop signals' for the termination of polypeptide chain synthesis during translation.
        - The stop codon does **not** code for any amino acid.

4. The genetic code is **non-overlapping** - the **reading frame** for each triplet is **fixed**.

    - The codons do not overlap i.e. each nucleotide in a codon is only used once.

        - A - A - U - C - G - C - is non-overlapping √
        - A - A - U - C - G - C - is overlapping X

5. The genetic code is **universal** i.e. it is **used in all organisms**.
    - With some minor exceptions, in all organisms (both prokaryotes and eukaryotes), the same codon codes for the same amino acid.

Second letter

First letter	U	C	A	G	Third letter
U	UUU, UUC } Phe UUA, UUG } Leu	UCU, UCC, UCA, UCG } Ser	UAU, UAC } Tyr UAA Stop UAG Stop	UGU, UGC } Cys UGA Stop UGG Trp	U C A G
C	CUU, CUC, CUA, CUG } Leu	CCU, CCC, CCA, CCG } Pro	CAU, CAC } His CAA, CAG } Gln	CGU, CGC, CGA, CGG } Arg	U C A G
A	AUU, AUC, AUA } Ile AUG Met	ACU, ACC, ACA, ACG } Thr	AAU, AAC } Asn AAA, AAG } Lys	AGU, AGC } Ser AGA, AGG } Arg	U C A G
G	GUU, GUC, GUA, GUG } Val	GCU, GCC, GCA, GCG } Ala	GAU, GAC } Asp GAA, GAG } Glu	GGU, GGC, GGA, GGG } Gly	U C A G

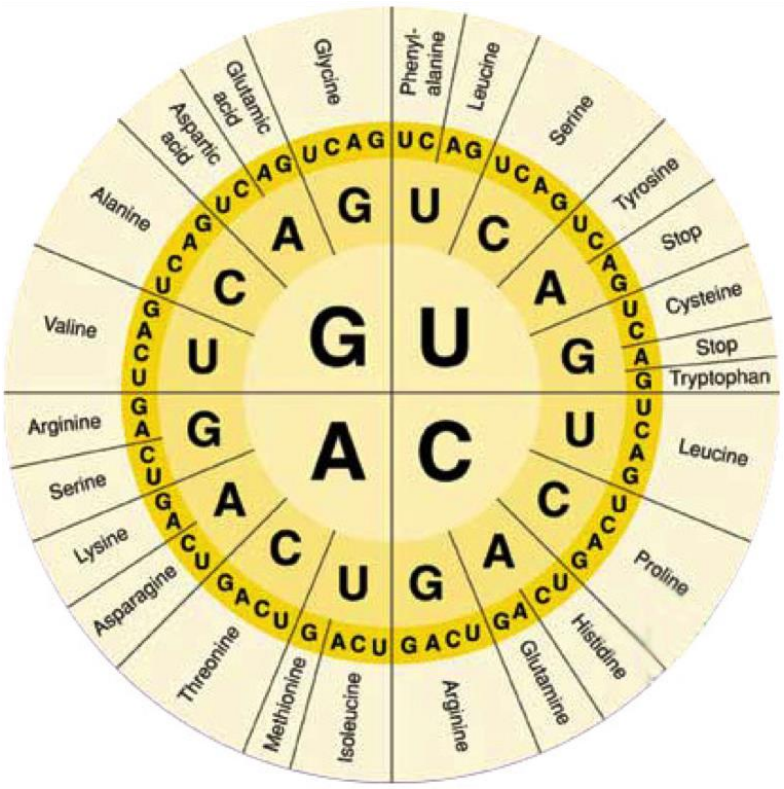
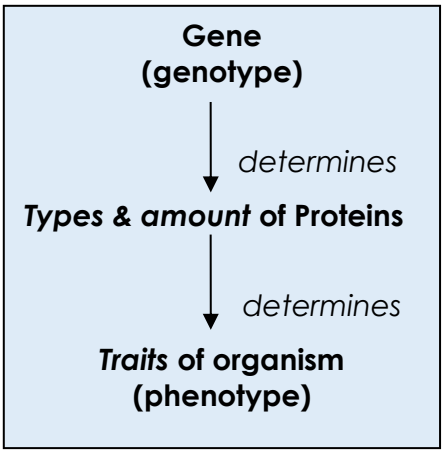


Fig. 3.2b: The genetic code – Different forms of representations of codon tables for mRNA to amino acid

3.3 Gene expression



- The trait of an organism (phenotype) is determined mostly by the **types** and/or the **amounts** of **proteins** produced. Proteins produced can be enzymes, hormones or other proteins.

- The type and the amount of proteins produced are in turn determined by the genes (genotype).

- Therefore, **gene expression** refers to the **synthesis of proteins** based on the information **encoded in gene sequences**.

- **Gene expression** involves 2 stages (Fig. 3.3a):
  1. **Transcription** – The **synthesis of mRNA** using DNA as template.
  2. **Translation** – The **synthesis of polypeptides** using mRNA as template.

- Expression of some genes does not involve translation. i.e. transcription of tRNA genes and rRNA genes form tRNA and rRNA respectively, which are then **not** translated.

- Only protein-coding genes are transcribed into mRNA, which is then translated into polypeptides containing a specific sequence of amino acids determined by the gene sequence.

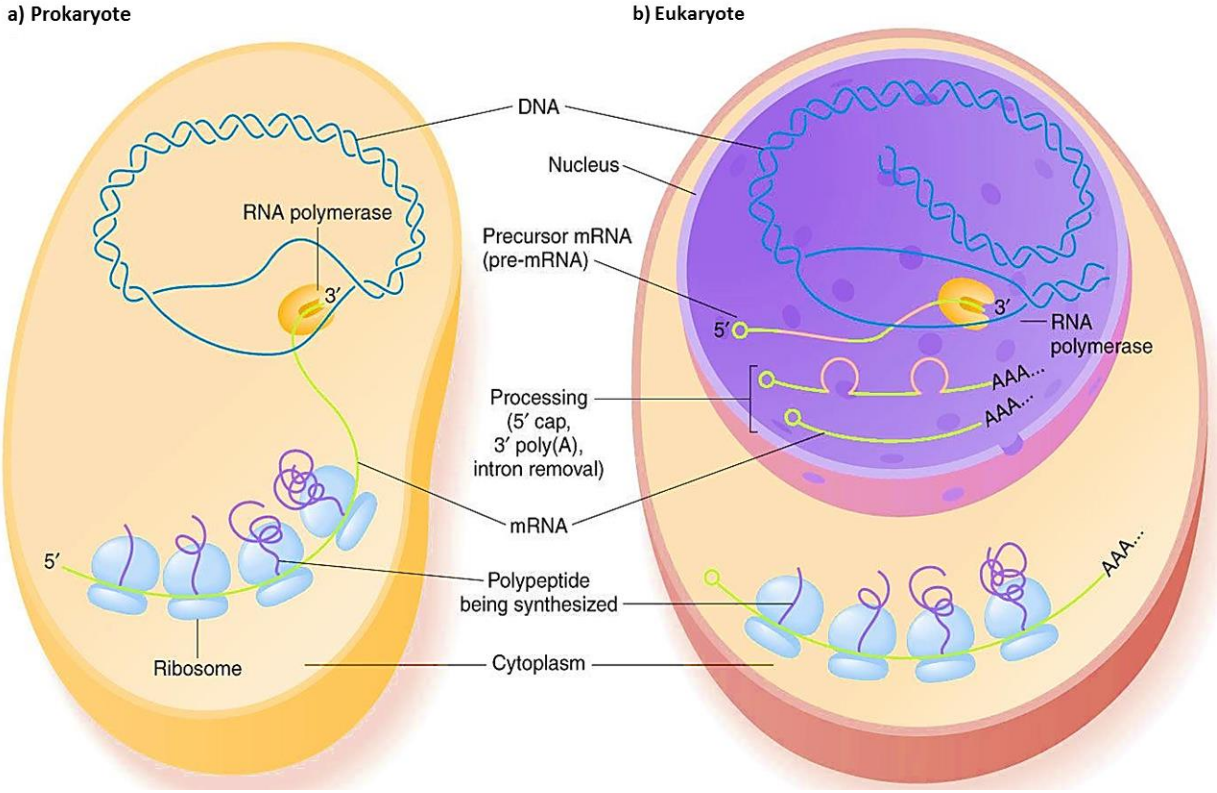


Fig. 3.3a: Overview of gene expression in (a) prokaryotes and (b) eukaryotes.
In bacteria, due to the lack of nucleus, transcription and translation occur simultaneously. As soon as mRNA is produced during transcription, translation of the mRNA takes place. In contrast, in eukaryotic cells, transcription occurs in the nucleus to produce mRNA which is then transported out into the cytoplasm for translation.

3.3.1 Transcription

- **Transcription** is the **synthesis of RNA (mRNA, tRNA and rRNA) by using the DNA template stand** (Fig. 3.3b).

- The synthesis of RNA begins in a living **cell that is <u>not</u> undergoing cell division**.

- Transcription takes place in the **nucleus** of **eukaryotic cells** but in the **cytoplasm** of **prokaryotes**.

- The segment of DNA where transcription occurs is known as a **transcription unit** and comprises 3 main regions: (Fig. 3.3b)

  a. Promoter
  b. RNA-coding region, which includes the
  c. Terminator

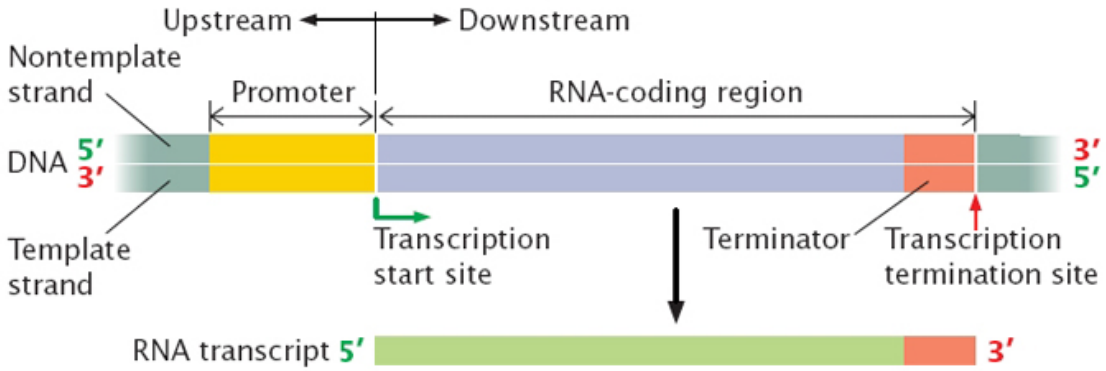


Fig. 3.3b: Three main regions of a transcription unit

Is there a difference between a gene and a transcription unit?

- Not really. The term "gene" is more commonly used and generally associated with the concept of heredity.
- The term "transcription unit" is more technical and generally used when describing the process of transcription.

a. Promoter

- Promoter is the region of **DNA upstream** of the RNA-coding region, where **RNA polymerase** and a collection of proteins called **transcription factors** attach to and initiate transcription.

- In **<u>eukaryotes</u>**, there is a DNA sequence **within the promoter** known as the **TATA box**.

  - The TATA box is recognized by **transcription factors** and **RNA polymerase**.
  - The TATA box is named as such because it is rich in adenine (A) and thymine (T).

- In **<u>prokaryotes</u>**, the DNA sequence within the promoter recognized by only **RNA polymerase** is known as the **Pribnow box**.

b. RNA-Coding Region

- This is the region of **DNA** that is **transcribed** into an RNA molecule.
- In **<u>eukaryotes</u>**, there are coding and non-coding regions within the transcription unit.
  - **Coding regions** are known as **exons**, which are DNA sequences that are eventually expressed, usually by being translated into amino acid sequences.
  - **Non-coding regions** are known as **introns**, which are segments of DNA that lie between coding regions. Introns are transcribed but are not translated into amino acid sequences.
- In **<u>prokaryotes</u>**, there are **only coding regions** within the transcription unit.

c. Terminator

- The sequence of DNA **downstream** of the transcription start site that **signals the end of transcription**.

- Transcription consists of 3 main steps, namely (Fig. 3.3c):
  1. Transcription Initiation
  2. Elongation
  3. Termination

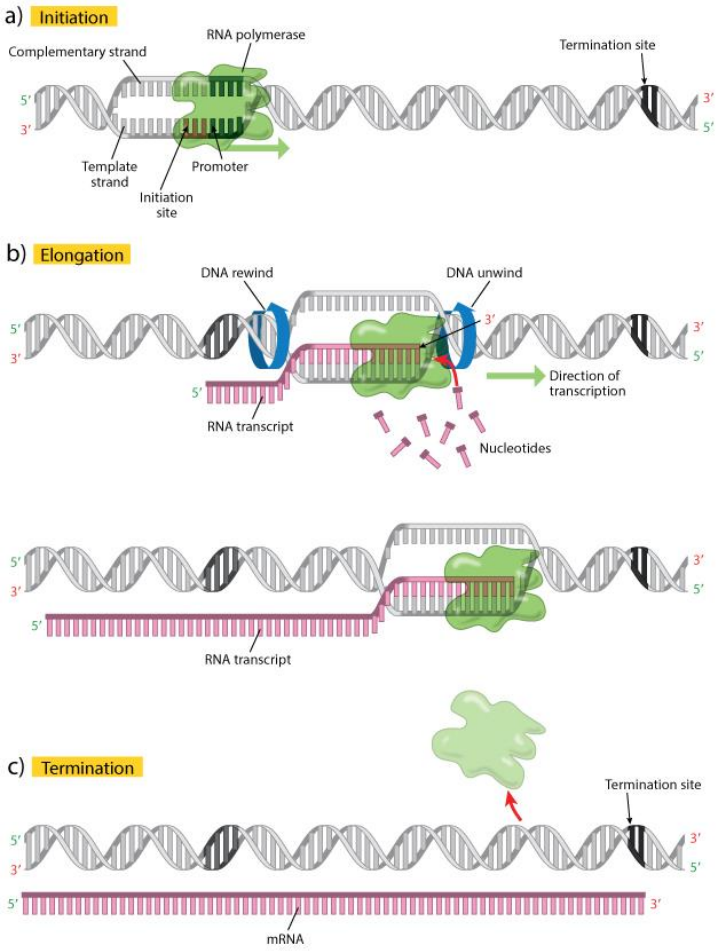


Fig 3.3c Shows an overview of the transcription process

1. Transcription initiation

In Eukaryotes (Fig. 3.3d)**:**

- First, a collection of proteins called **transcription factors** recognize and attach to the **TATA box** in the **promoter**.
- The **bound transcription factors facilitate** the **binding of RNA polymerase** to the **promoter**.
- The whole complex of transcription factors and RNA polymerase bound to the promoter is called a **transcription initiation complex**.
- **RNA polymerase** undergoes a **conformational change** and this allows it to be bound in the **correct orientation**.
- The RNA polymerase will then **unwind** and **unzip the DNA molecule** and starts **transcribing** the **template strand**.

In Prokaryotes:

- In prokaryotes, only **RNA polymerase** recognizes and binds to the **Pribnow Box in the promoter** to initiate transcription.

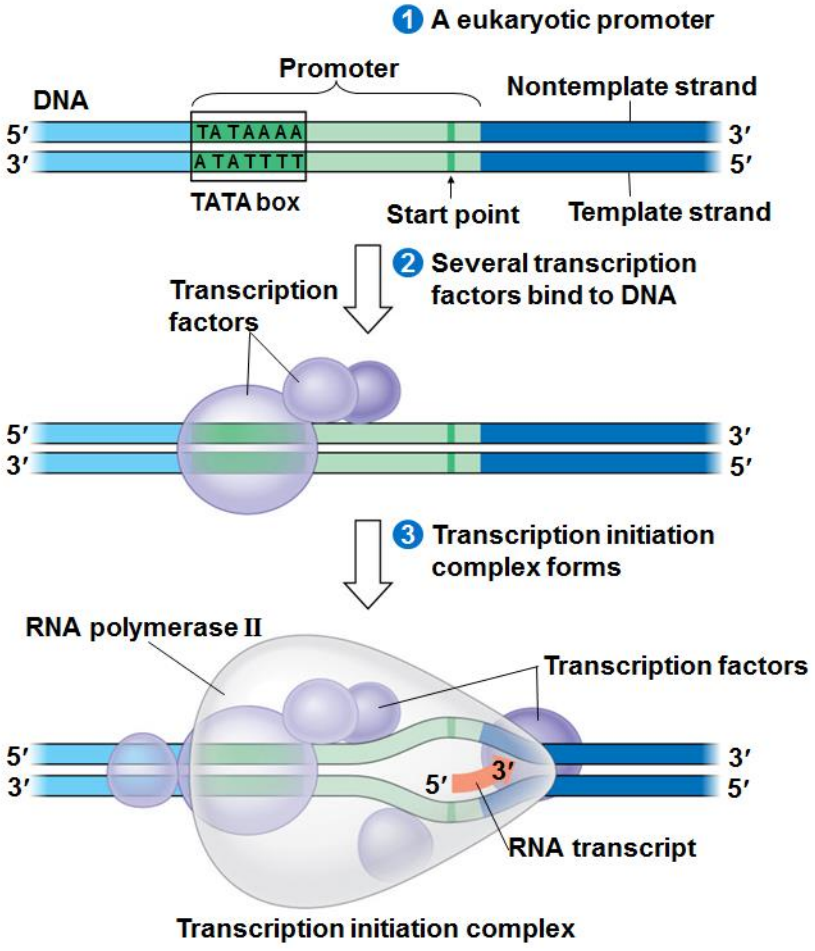


Fig. 3.3d: Initiation of Transcription in Eukaryotes

2. <u>Elongation</u> (Fig. 3.3e)

<u>In both Eukaryotes and Prokaryotes:</u>

- RNA polymerase **moves along** the **<u>DNA template strand</u>** in the **3' to 5'** direction and takes up **free ribonucleotides** which are complementary to the bases on the DNA template strand and matches them by **complementary base pairing**:
    - Adenine in DNA with **Uracil** in RNA
    - Thymine in DNA with Adenine in RNA
    - Cytosine in DNA with Guanine in RNA
    - Guanine in DNA with Cytosine in RNA

- RNA polymerase then catalyses the formation of the **phosphodiester bonds** between **adjacent ribonucleotides**.

- Unlike DNA polymerase, RNA polymerases **<u>does not require</u>** an existing free 3'OH group to add ribonucleotides. Hence, **no primer** is **needed**.

- Since the nucleotide sequence on the **DNA template strand** is **read** in the **3' to 5' direction**, **messenger RNA is synthesised in the 5' to 3' direction** (due to the antiparallel nature of the 2 polynucleotide strands)**.**

- 5' to 3' elongation of the mRNA strand proceeds until RNA polymerase reaches the terminator region.

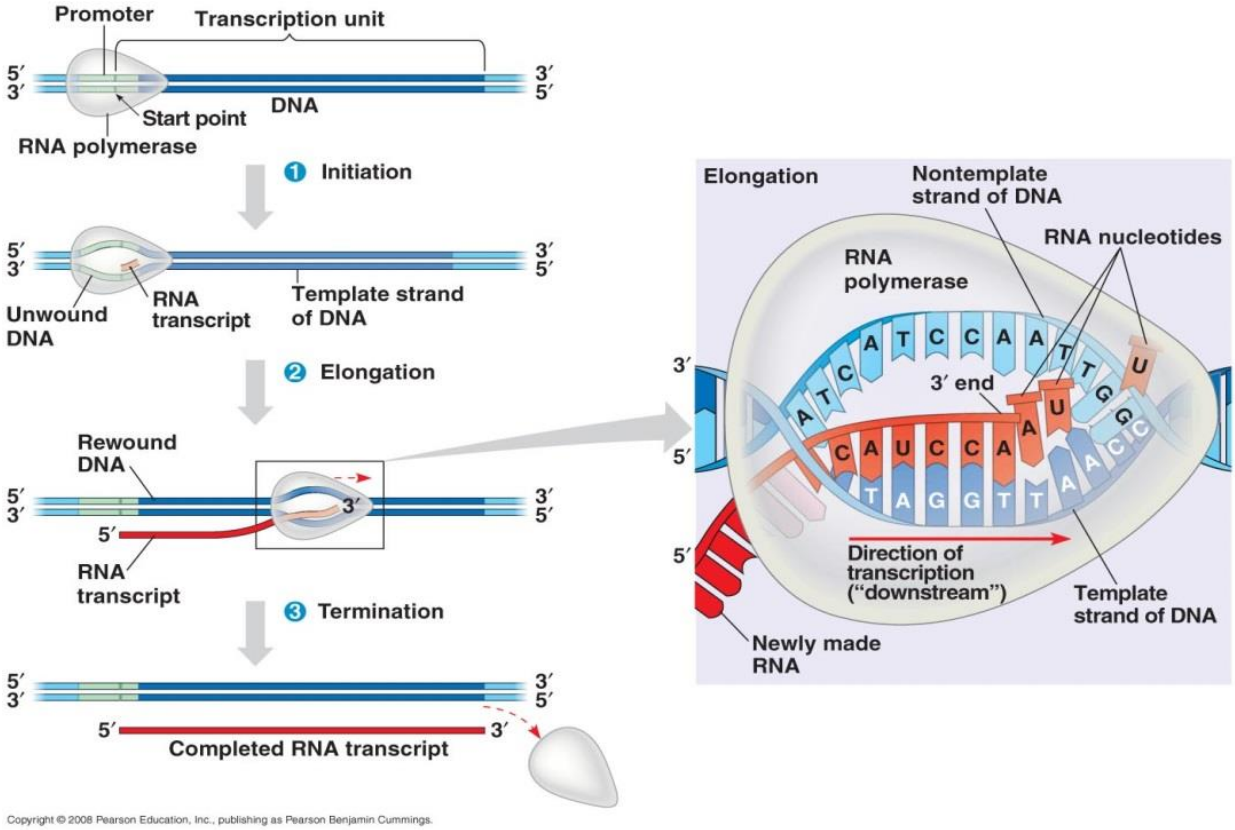


Fig. 3.3e: Elongation of RNA transcripts.

3. **Termination**

In Eukaryotes:

- Termination of transcription occurs within a **terminator region**. However, there is **no well-defined sequence** within this region.

- Transcription is terminated only **after RNA polymerase transcribes the DNA sequence** (TTATTT) which **codes for a polyadenylation signal** (AAUAAA) (Fig 3.3f).

- This is usually about 10 to 35 nucleotides downstream of the polyadenylation signal.

- **Proteins** associated with the growing RNA strand will cause the **RNA polymerase** to **detach from the DNA**, thus also releasing the RNA strand.

- The product of transcription is known as an **RNA transcript** (general term).

  If the RNA formed is mRNA, it is known as a **pre-mRNA** molecule (Fig. 3.3f), which consists of:

  - Exons and introns
  - 5' UTR (a short region before the start codon)
  - 3' UTR (short region at the end of the last exon, which includes the polyadenylation signal)

- The regions 5' UTR (UnTranslated Region) and 3' UTR serve important functions (Refer to Topic 8):

  - The **5' UTR** facilitates the **binding of the small ribosomal subunit on the mRNA,** and hence helps to **increase the translational efficiency** of the mRNA.
  - The **3' UTR** helps to **maintain stability** of the mRNA.

**Note: The 5' UTR and 3' UTR are part of the first and last exon respectively.*

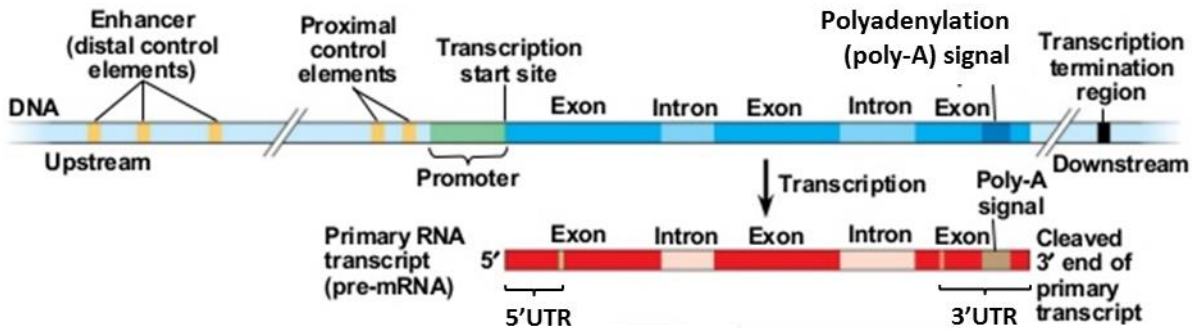


Fig. 3.3f Shows the position of the polyadenylation signal and transcription termination region on the DNA (blue). Also shows the positions of the polyadenylation signal and UTRs on the resultant pre-mRNA

In Prokaryotes

- In prokaryotes, RNA polymerase transcribes a **terminator sequence** in the DNA template strand. (Fig. 3.3g)
    - This terminator RNA sequence functions as a **termination signal**, causing the **RNA polymerase** to **detach** from the DNA and **release** the **RNA strand**.

- The resulting mRNA transcript consists of:
    - Protein-coding sequence starting with AUG (Note: there are no introns in prokaryotes)
    - 5' UTR (a short region before the start codon that is not translated)
    - 3' UTR (short region at the end of the last exon that is not translated)

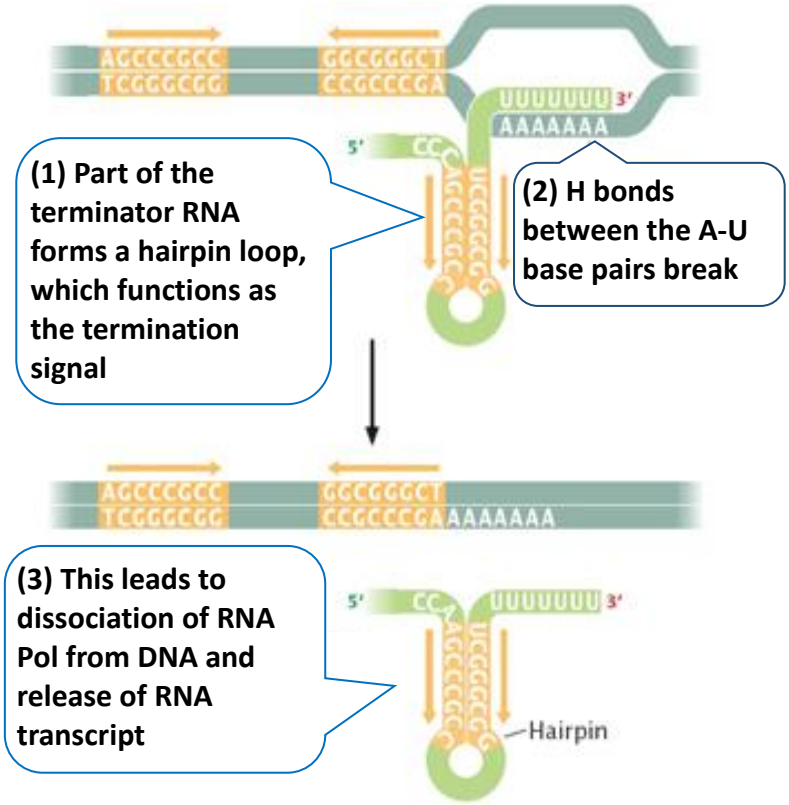


Fig. 3.3g: Termination of prokaryotic transcription.

3.3.2 Post-Transcriptional Modification (In Eukaryotes only)

In Eukaryotes

- In eukaryotes, there are other processes that occur ***after transcription*** and ***before*** **translation**.
  - These regulatory processes are collectively termed **post-transcriptional modifications**.

- An mRNA molecule that has **not completed** post-transcriptional modification is termed **pre-mRNA** / primary mRNA transcript.

- An mRNA molecule that has **completed** its post-transcriptional modification is termed **mature mRNA**.

- In **eukaryotic cells**, after **transcription** occurs in the **nucleus**, the **mRNA** has to be **transported out** of the nucleus through the **nuclear pore** for translation to occur in the cytoplasm.

- **To facilitate the transport of mRNA** and **regulation of gene expression**, post-transcriptional modification must first occur in the **nucleus**. The **pre-mRNA undergoes** the following post-transcriptional modifications (Fig.3.3h; more details will be covered in Topic 8):

  1. **Addition of 5' modified GTP Cap**

     - A **modified guanosine triphosphate (GTP)** is added to the 5' end of the pre-mRNA.
     - The addition of the 5' GTP Cap (i) facilitates the export of mature mRNA, (ii) protects the mRNA from degradation and (iii) helps ribosomes attach to the 5' end of the mRNA during translation.

  2. **RNA Splicing**

     - **Introns** (segments of the RNA-coding region that will not be translated) are **excised** (i.e., removed)
     - **Exons** (segments of the RNA-coding region that will be translated) are **spliced** (i.e., joined together)

  3. **Addition of 3' Poly (A) Tail**

     - A chain of **adenine** nucleotides is added to the 3' end of the pre-mRNA.
     - Like the modified 5' GTP cap, the 3' Poly (A) tail (i) facilitates the export of mature mRNA, (ii) protects the mRNA from degradation and (iii) helps ribosomes attach to the 5' end of the mRNA during translation.

- At the **end of post-transcriptional modification**, the mRNA transcript is termed **mature mRNA** and it consists of (Fig. 3.3h):

  - 5' GTP Cap
  - 5' untranslated region (5'UTR)
  - Coding sequence consisting of exons only
  - 3' untranslated region (3'UTR)
  - 3' Poly adenylated tail (Poly-A tail)

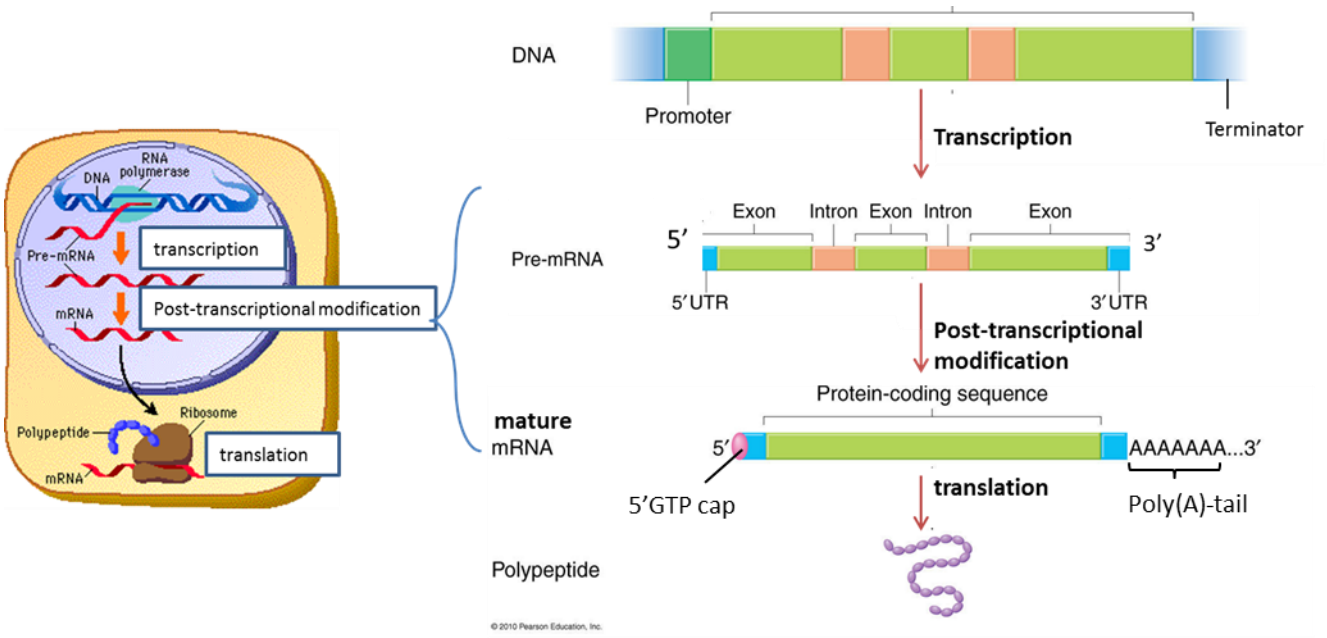


Fig. 3.3h: Post-transcriptional modification of a pre-mRNA molecule shown. Note the regions of a mature mRNA in eukaryotes.

Post-transcriptional modification can only start after transcription has completed. True or False?

In Prokaryotes

- Prokaryotic mRNA does not contain introns. Hence, prokaryotes **do not undergo post-transcriptional modification**.
- Since there is also no nucleus in prokaryotic cells, the transcribed mRNA in the cytoplasm can be used directly for translation.

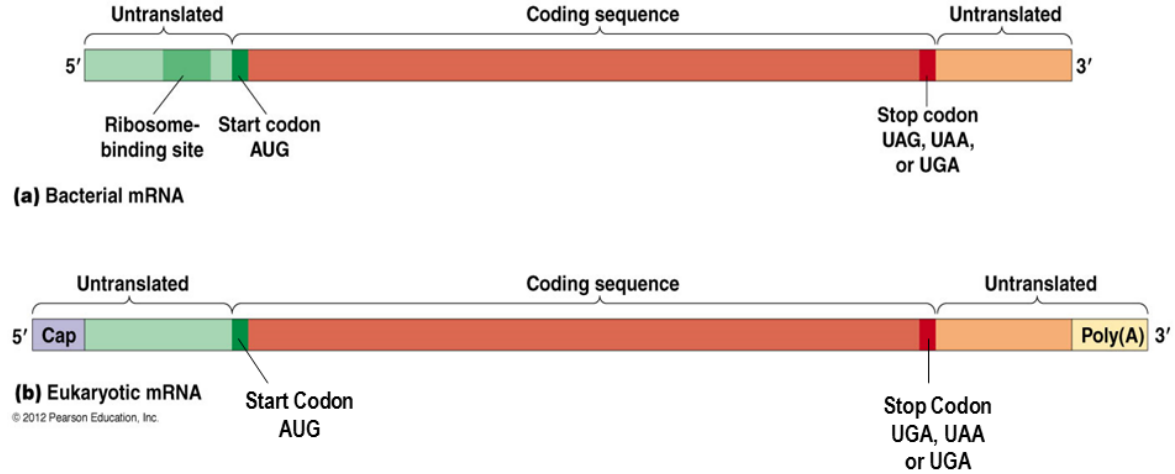


Fig. 3.3i: Structural features of Bacterial (Prokaryotic) mRNA and Eukaryotic mature mRNA

3.3.3 Translation

- Translation is the **synthesis of a specific <u>sequence</u> of amino acids** (which forms the polypeptide chain) using the **base sequence on an mRNA template**. (Fig. 3.3j).

- Besides mRNA, **tRNA** and **ribosomes** (comprising **rRNA** and **ribosomal proteins**) also take part in translation. (Recall Topic 2 on details of their structures)

- The process of translation is similar for both eukaryotes and prokaryotes.

- However, in **eukaryotes**, it is the **mature mRNA** that is translated.

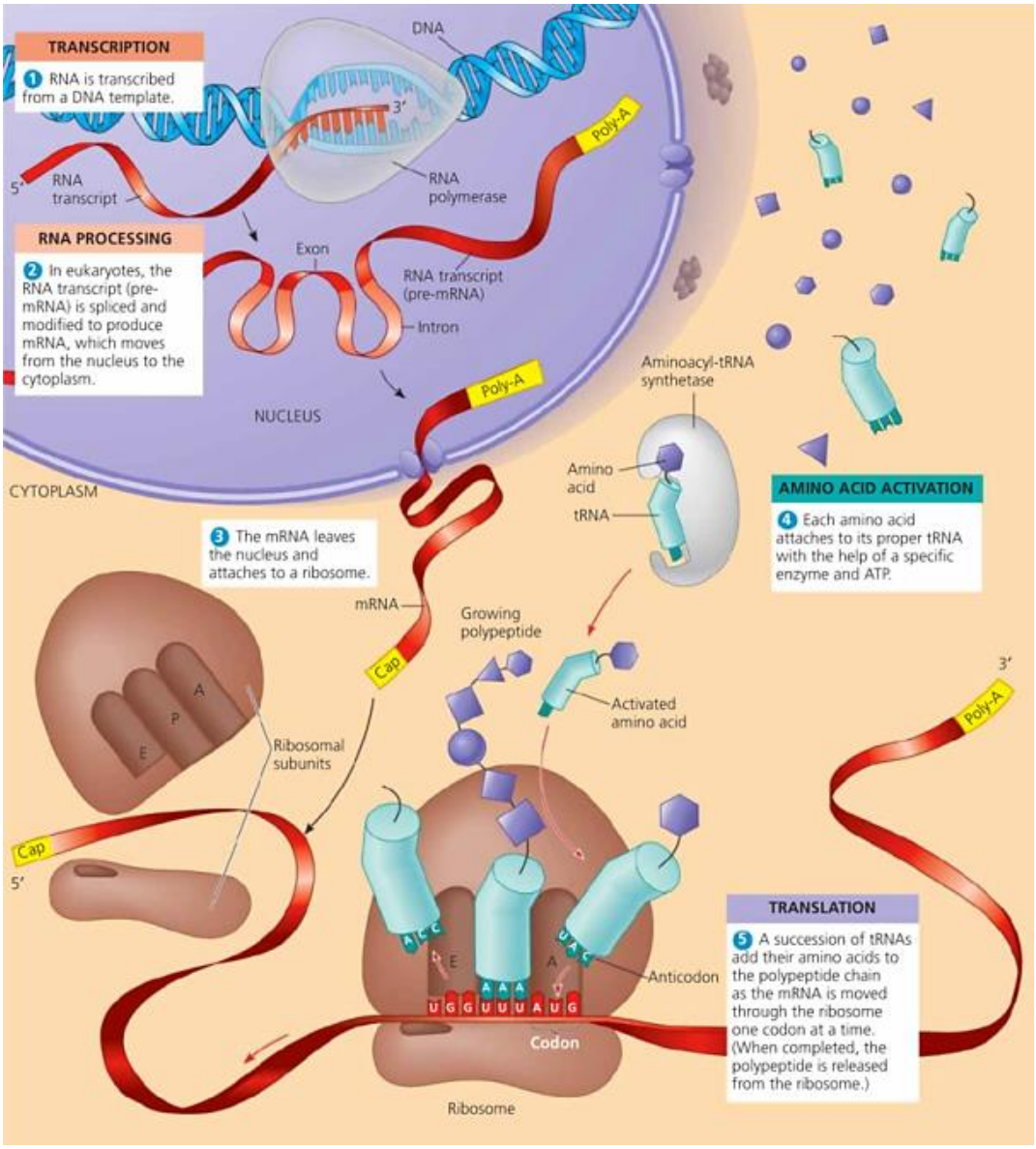


Fig. 3.3j Shows the locations and sequence of (1) transcription, (2) RNA processing, (4) amino acid activation and (5) translation.

- Before the translation can start, **amino acid activation** must first occur: (Fig. 3.3m)
    - **Free amino acids** in the cytoplasm **must be joined to a specific tRNA** before they can be incorporated into a growing polypeptide chain.
    - *Recall:* The **function of tRNA** is to **transfer amino acids to** the **ribosomes for translation**.
    - Each tRNA contains a **3' CCA end** which serves as an **amino acid attachment site** (Fig. 3.3k)

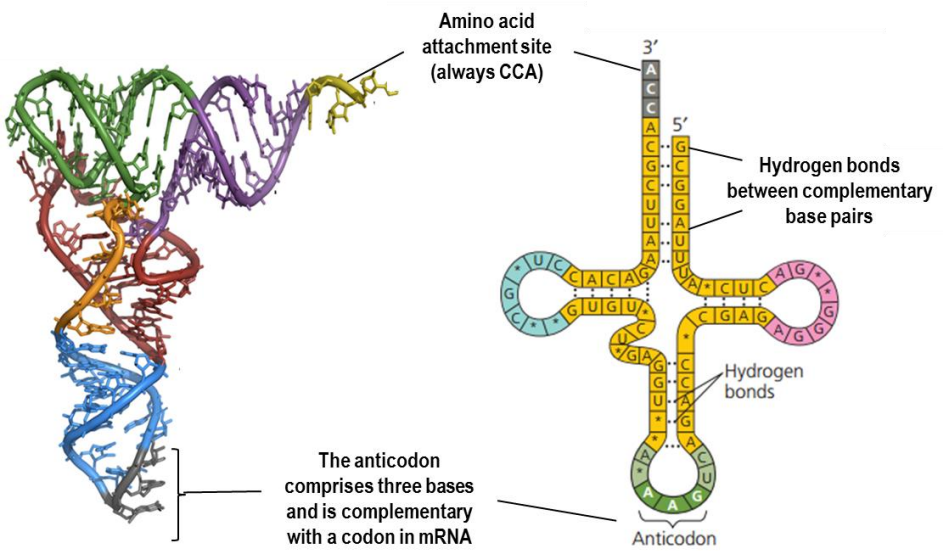


Fig. 3.3k: The tRNA structure as shown in 3D (left) and 2D (right).
Note the positions of the anticodon and the amino acid attachment site.

- Amino acid activation is catalyzed by the enzyme **aminoacyl-tRNA synthetase**. (Fig. 3.3l and Fig. 3.3m)
- There are at least **20 different** aminoacyl-tRNA synthetases, one for each amino acid.
- The active site of aminoacyl-tRNA synthetase consists of the
  (1) amino acid-binding site and
  (2) anticodon-attachment site (Fig 3.3l).

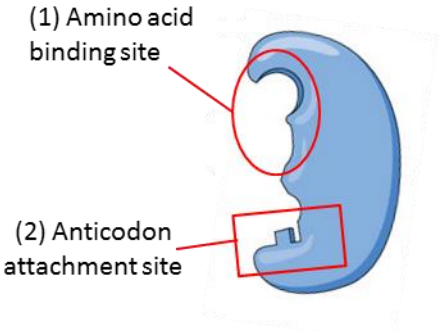


Fig. 3.3l Shows the active site of aminoacyl-tRNA synthetase

- Process of amino acid activation:

1. An **amino acid** and **ATP** molecule bind to the **specific amino acid binding site** of the aminoacyl-tRNA synthetase due to their **complementary shapes**.

2. The enzyme catalyzes the formation of an **aminoacyl-AMP-enzyme complex** by removing two phosphate groups from ATP and joining the amino acid to the remaining AMP.

3. A **specific tRNA** then attaches to the complex due to the **<u>complementary shapes</u>** of the **tRNA anticodon** and its **anticodon attachment site**.

   The amino acid is then attached to the last nucleotide of the 3' CCA end of the tRNA, forming an **aminoacyl-tRNA complex**.

4. The **aminoacyl-tRNA complex** is then **released** from the enzyme and used for translation. The amino acid is now activated.

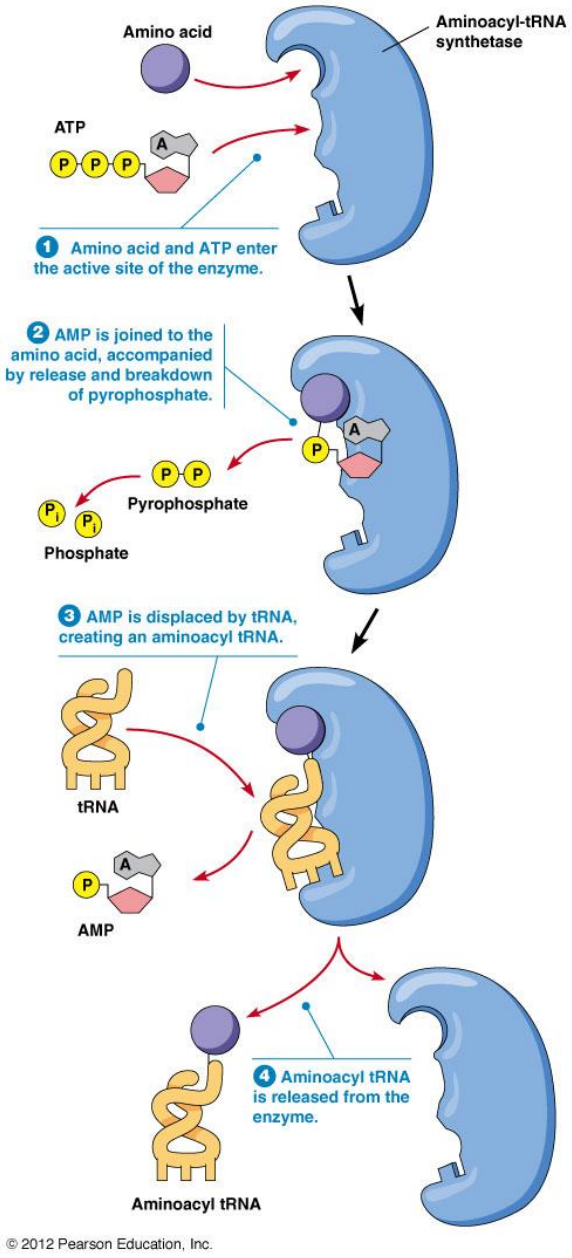


Fig. 3.3m: Process of amino acid activation

What is/are the substrate(s) for aminoacyl-tRNA synthetase?

1. <u>Initiation of Translation</u> (Fig. 3.3n)

- When translation begins, the **small subunit of the ribosome** binds to the **5' end** of the mRNA molecule and moves in the direction of the 3' end to scan for the start codon (AUG).
- An **initiator tRNA** with an **anticodon UAC** undergoes **complementary base pairing** with the **start codon AUG on mRNA** via hydrogen bonds.
- This **initiator tRNA** also **carries** the amino acid **methionine** at its 3' CCA end.
- The **arrival of** a **large ribosomal subunit** then completes the **translation initiation complex**. The initiator tRNA is now also bound to the **P site of the ribosome.**
- **The large ribosomal subunit** has three binding sites:
    - **Aminoacyl-tRNA binding site (A),**
    - **Peptidyl-tRNA binding site (P),**
    - **Exit site (E)**.
- **GTP** provides the **energy** for the assembly.

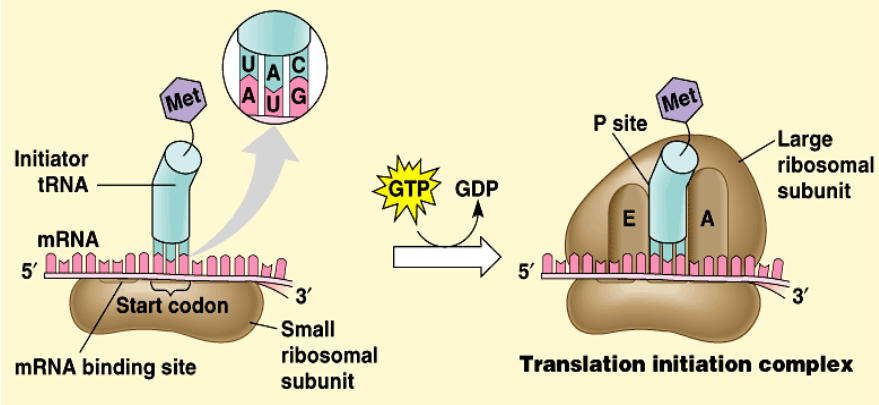


Fig. 3.3n: Initiation of translation.

Since the initiator tRNA always carries methionine, does it mean that methionine is always the first amino acid in all proteins?

Is there only one AUG codon in every mRNA?

2. <u>Elongation</u> (Fig. 3.3o)

- At this point, the **A-site** is **available** for the next aminoacyl-tRNA.
- The **aminoacyl-tRNA with an anticodon complementary** to the **second codon** enters the **A site**.
- **Peptidyl transferase,** a **ribozyme** within the **P site** of the large ribosomal subunit, then **catalyses** the formation of a **peptide bond between** the first **two amino acids**.
- Once the peptide bond is formed, the **ribosome moves (translocation) along the mRNA from the 5' to 3' direction to the next codon.** The tRNA that was in the P site now occupies the E site and the tRNA which was in the A site now occupies the P site.
- The first **tRNA in** the **E site** is no longer attached to an amino acid and **exits the ribosome** to be reactivated.
- The third aminoacyl-tRNA complex with an anticodon complementary to the third codon enters the A site.
- This **process is repeated** until a **stop codon** is reached.

NOTE: The catalytic function in ribosome is due to rRNA (ribozyme), not proteins.

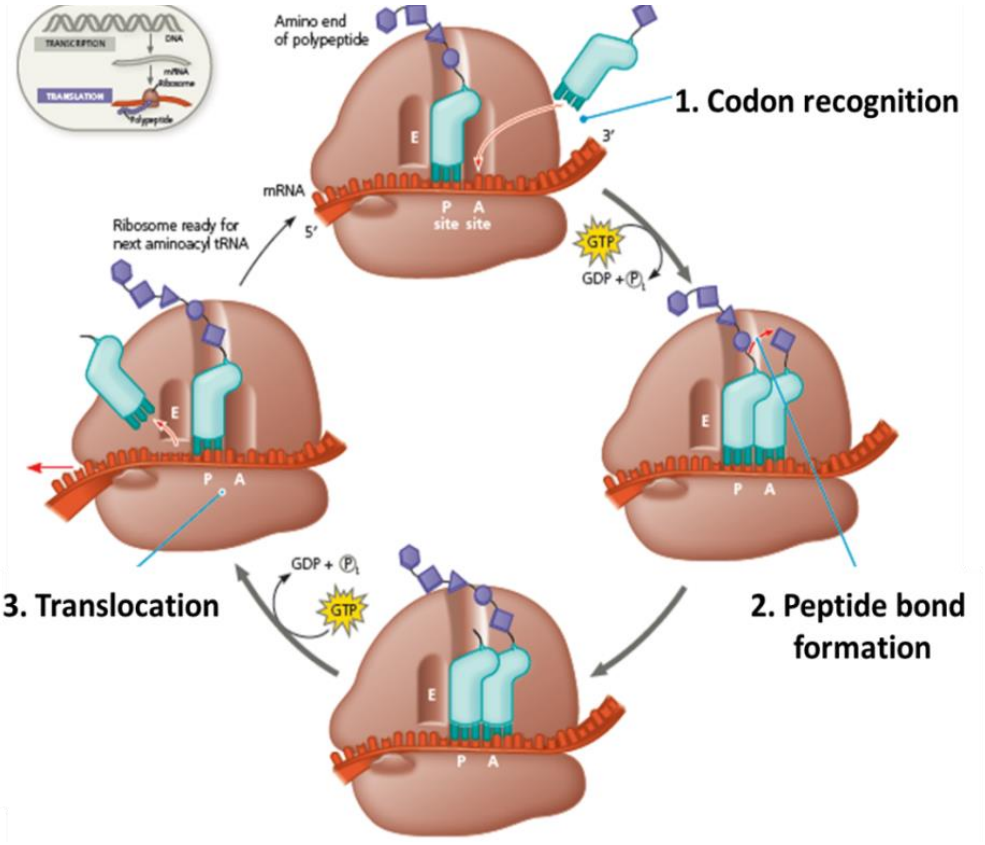


Fig. 3.3o: Elongation in translation.

3. <u>Termination</u> (Fig. 3.3p)

- When the A site of the ribosome reaches a **stop codon** (**UAA UGA** or **UAG**) on the mRNA, a protein known as the **release factor** binds to the stop codon.

- The **release factor** recruits **a water molecule,** instead of an amino acid, to the polypeptide chain.

- This **hydrolyses the bond between the completed polypeptide and the last tRNA**, which frees the polypeptide chain and causes the translation complex to dissociate.

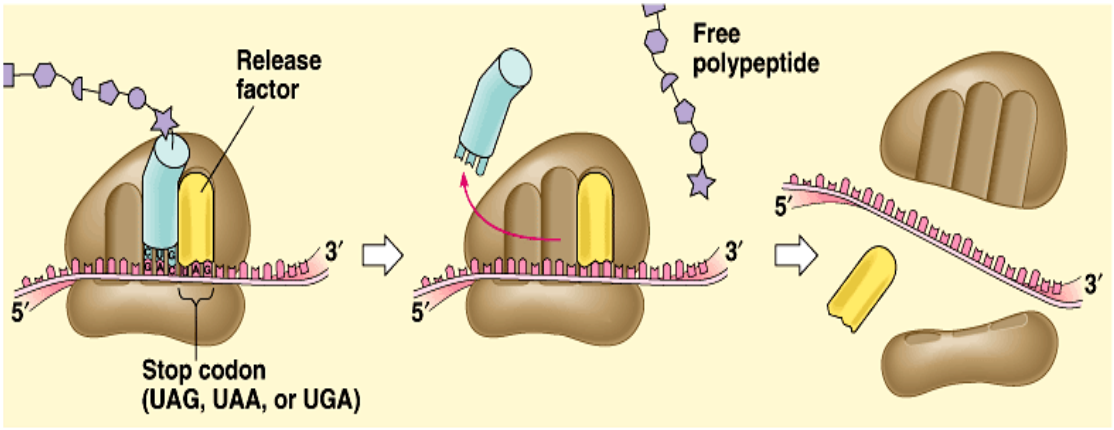


Fig. 3.3p: Termination of translation**.**

3.3.4 Post-Translational Modification

- The newly synthesized **polypeptide coils** and **folds** into its **three-dimensional shape** spontaneously, from primary structure to the secondary and/or tertiary structure, to become a **functional protein**.

- Some proteins may require further **post-translational modifications** before becoming functional.

- Addition of carbohydrates, lipids, or phosphate groups to the polypeptide are among the many post-translational modifications that may take place in the rough endoplasmic reticulum and the Golgi apparatus in eukaryotes, and in the cytoplasm in prokaryotes.

3.4 Summary: Roles of RNAs in protein synthesis

1.	**mRNA**	• The mRNA molecule is formed from the transcription of a specific region of DNA (known as genes) which codes for the polypeptide. • mRNA acts as a **carrier molecule, conveying the genetic message from the nucleus to the ribosomes in the cytoplasm.** • Each mRNA molecule has a sequence of nucleotides. Each triplet of nucleotides (codon) codes for one amino acid. • **mRNA codons specify the order in which amino acids are sequenced** to form a polypeptide.
2.	**tRNA**	• The **CCA site** at the **3' end** allows for the **attachment of an amino acid**. • The tRNA molecule functions to **carry an amino acid to the ribosome during protein synthesis**. • Each tRNA molecule is **specific** – it has a **specific sequence on its anticodon** and carries its **corresponding amino acid**. • Every tRNA has an **anticodon** that is **complementary** to the **codon on the mRNA**. • This allows for the **correct sequencing of amino acids** on the polypeptide chain coded for by the gene.
3.	**rRNA**	• During translation, rRNA acts as an enzyme (ribozyme) providing **peptidyl transferase activity**, **catalyzing the formation of peptide bonds between amino acids**. • rRNA has a **structural role** in helping to **orientate the ribosomal proteins into the correct position within the ribosome**. • **rRNAs from the large and small subunits** interact, aiding **in the binding of the two** subunits during translation. • **Stabilizes** the **interaction between mRNA and tRNA**.

4. Comparisons

4.1 Comparison between Transcription and Translation

FEATURE	TRANSCRIPTION	TRANSLATION
Location		
Template	DNA template strand	mRNA
Enzyme	_____ catalyses the formation of phosphodiester bonds between adjacent ribonucleotides	Formation of peptide bonds between adjacent amino acids catalysed by catalytic site present in the _____ of the _____ (consists of 2 subunits of protein & RNA molecules)
Bond between basic units	A _____ is formed between the 5' phosphate group of the incoming nucleotide and 3'OH group of the growing RNA transcript.	_____ are formed between adjacent amino acids.
Direction of Reading of template	RNA polymerase moves along and reads the DNA template strand in a _____ direction.	Ribosomes move along and read the mRNA molecule from the _____ end.
Raw material(s)	Free ribonucleotides / ribonucleoside triphosphates. These are lined up in a sequence complementary to that on the DNA template strand.	_____. These have to first be activated by attaching them to their specific tRNA molecules.
Product(s)	_____, _____, _____ All exit the nucleus via the nuclear pores for their respective functions in the cytoplasm.	

4.2 Comparison between DNA replication and Transcription

Similarities:

- Both occur within the nucleus.
- Complementary base pairing occurs.
- Unzipping of DNA double helix occurs.
- Parental DNA strands separate progressively in short segments.

Differences:

FEATURE	DNA REPLICATION	TRANSCRIPTION
Enzyme involved		
Raw materials	Deoxyribonucleoside triphosphate	
Template	_____ strands of the DNA molecule act as the template The whole DNA molecule is being replicated	Only the _____ strand of the DNA molecule acts as a template. Only specific genes which have been activated are transcribed.
Base pairing	Adenine with _____ Cytosine with _____	Adenine on DNA with _____ on RNA Thymine on DNA with _____ on RNA Cytosine with guanine
Proofreading property	_____ proofreads the newly synthesized daughter strand, ensuring precise complementary base pairing.	No proofreading property in RNA polymerase
Product(s)	2 DNA molecules. Each DNA molecule comprises of 1 parental strand and 1 complementary daughter strand. Products remain in the nucleus.	_____, _____, _____ All exit the nucleus via the nuclear pores for their respective functions in the cytoplasm.

4.3 Comparison between DNA replication and Translation

FEATURE	DNA REPLICATION	TRANSLATION
Location		
Monomers		Amino acids
No. of different monomers		20
Bond formed between monomers		Peptide bond
Template		mRNA
Direction of reading of template		5’ to 3’ direction
Product	2 DNA molecules. Each DNA molecule comprises of 1 parental strand and 1 complementary daughter strand. Products remain in the nucleus.	Products fold into their specific 3D conformation and may function in the cell or outside the cell.
Enzyme for elongation / bond formation between monomers	DNA polymerase	
Initiation	Origin of replication	
Termination	When DNA polymerase reaches the end of the parental strand	

☺ *End of Topic* ☺