Central dogma

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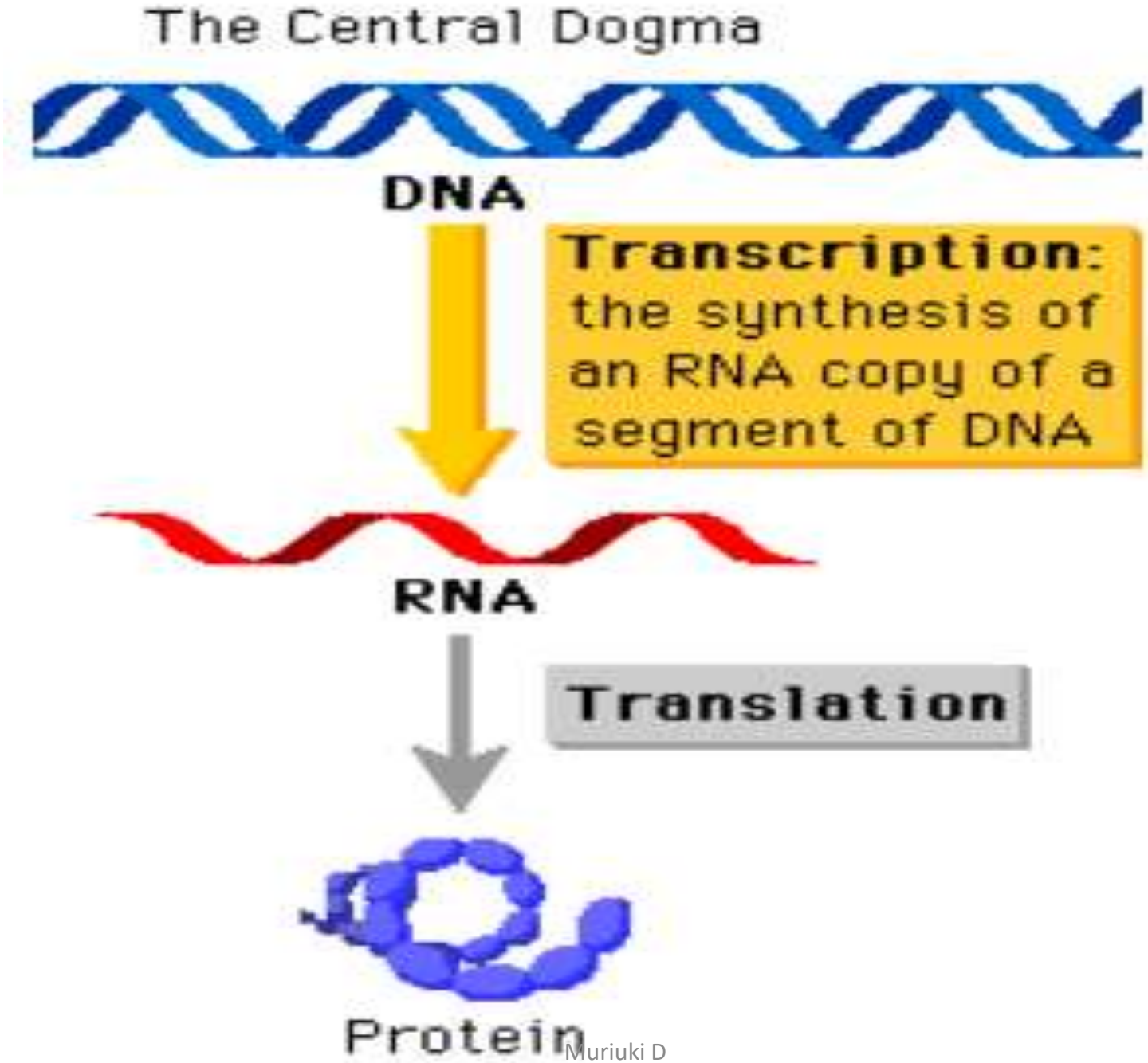
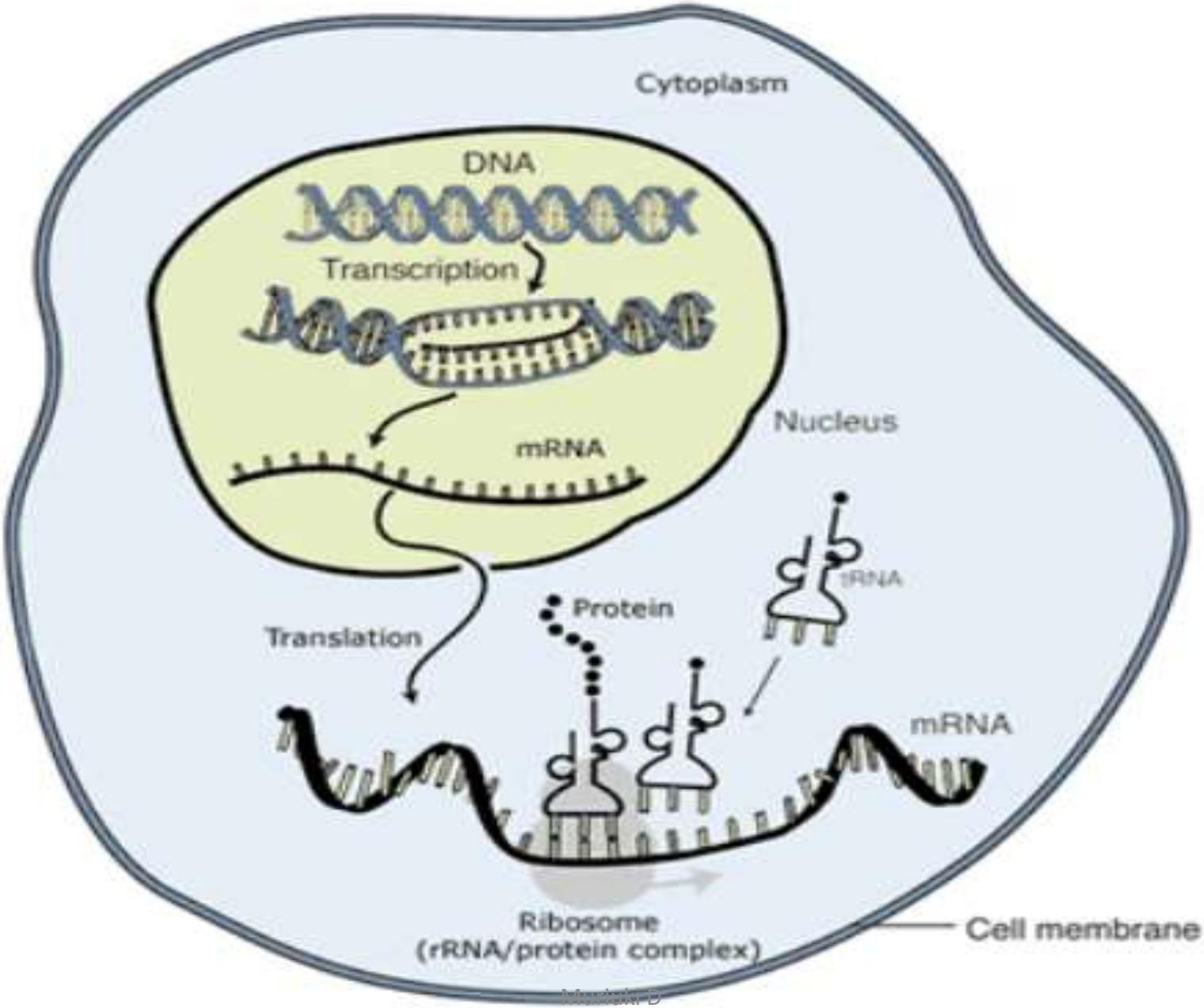

The Central Dogma
DNA
Transcription: the synthesis of an RNA copy of a segment of DNA
RNA
Translation
Protein

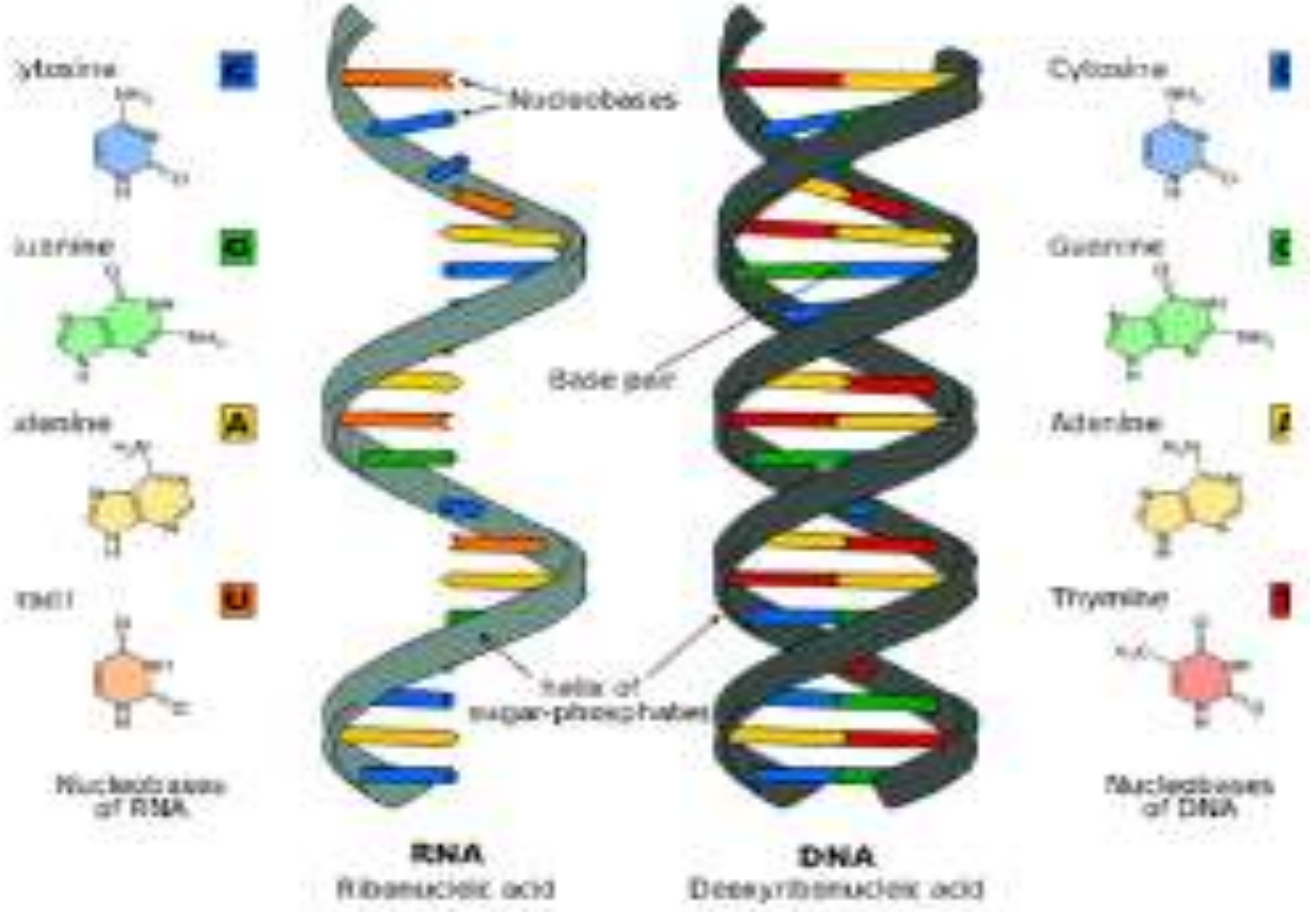

Cytoplasm
DNA
Transcription
Nucleus
mRNA
tRNA
Protein
Translation
mRNA
Ribosome
(rRNA/protein complex)
Cell membrane

Introduction

- Central dogma of molecular biology describes the two-step process, transcription and translation, by which the information in genes flows into proteins:

DNA → RNA → protein.

- The central dogma of molecular biology is a phrase by **Francis Crick**, who proposed the double helix structure of DNA.
- It means that information passes from DNA to proteins via RNA, but **proteins cannot** pass the information back to DNA.
- **Crick** first wrote it in 1958, and repeated it in 1970.

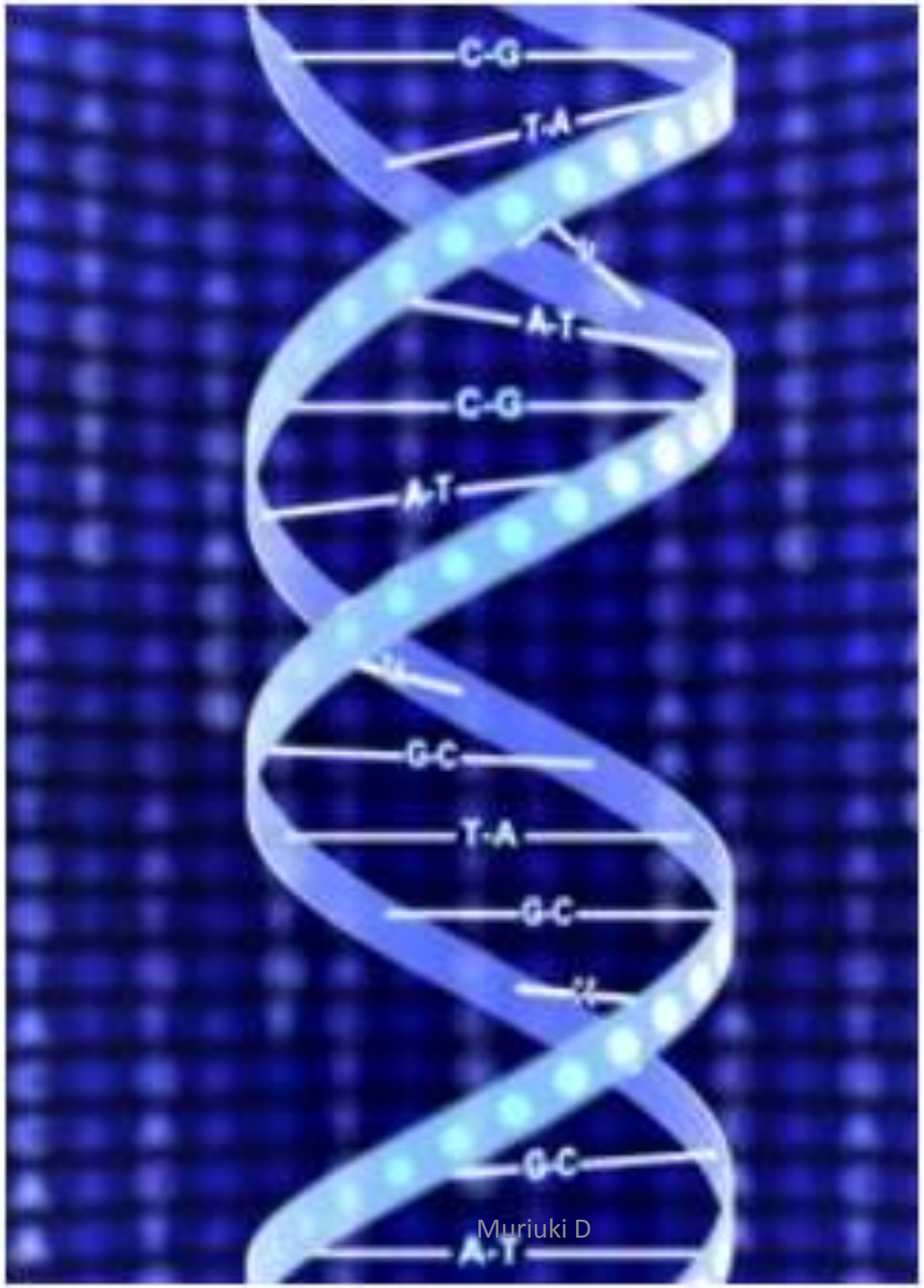


DNA vs. RNA: The Cells' Carriers of Genetic Information

DNA Replication

Enzymes necessary for DNA replication

- **DNA helicase** - unwinds and separates double stranded DNA as it moves along the DNA. It forms the replication fork by breaking hydrogen bonds between nucleotide pairs in DNA.
- **DNA primase** - a type of RNA polymerase that generates RNA primers. Primers are short RNA molecules that act as templates for the starting point of DNA replication.
- **DNA polymerases** - synthesize new DNA molecules by adding nucleotides to leading and lagging DNA strands.
- **Topoisomerase or DNA Gyrase** - unwinds and rewinds DNA strands to prevent the DNA from becoming tangled or supercoiled.
- **Exonucleases** - group of enzymes that remove nucleotide bases from the end of a DNA chain. Also proof-reads the newly synthesised strand
- **DNA ligase** - joins DNA fragments together by forming phosphodiester bonds between nucleotides



Process of replication

Has 4 steps

1. Pre-initiation
2. Initiation
3. Elongation
4. Termination

Pre-initiation

- DNA **helicase** enzyme disrupts the hydrogen bonding between base pairs to separate the strands into a Y shape known as the **replication fork**.
- DNA is directional in both strands, signified by a 5' and 3' end. This notation signifies which side group is attached the DNA backbone.
- The **5' end** has a phosphate (P) group attached, while the **3' end** has a hydroxyl (OH) group attached.

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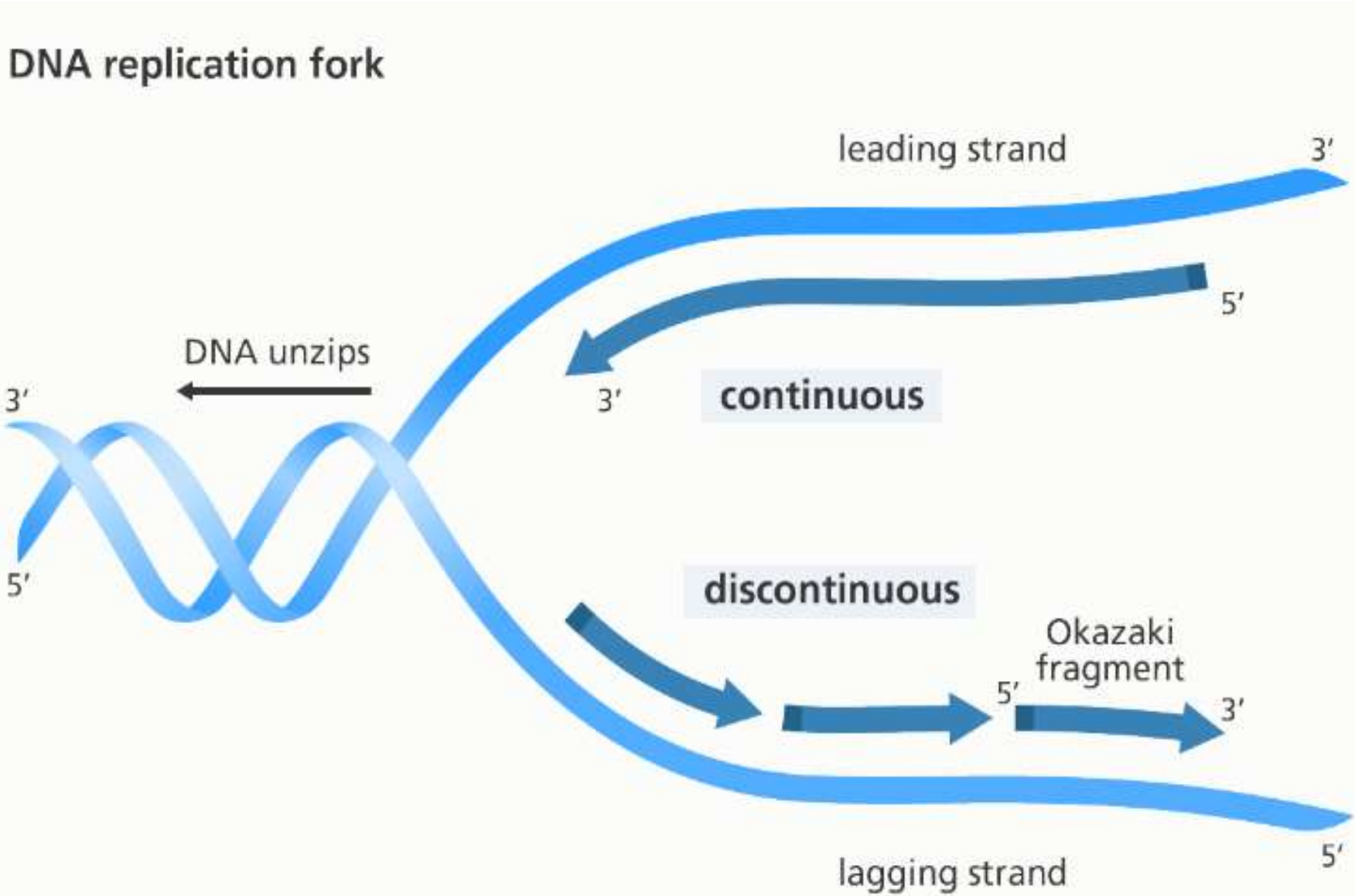
- This directionality is important for replication as it only progresses in the 5' to 3' direction.
- However, the replication fork is bi-directional; one strand is oriented in the 3' to 5' direction **(leading strand)** while the other is oriented 5' to 3' **(lagging strand)**.
- The two sides are therefore replicated with two different processes to accommodate the directional difference.



An illustration to show replication of the leading and lagging strands of DNA.

Image credit: Genome Research Limited

Initiation

- The leading strand is the simplest to replicate.
- Once the DNA strands have been separated, a short piece of RNA called a **primer** binds to the 3' end of the strand.
- The primer always binds as the starting point for replication.
- Primers are generated by the enzyme **DNA primase**.

Elongation

- **DNA polymerases** are responsible for creating the new strand by a process called elongation.
- There are five different known types of DNA polymerases in bacteria and human cells.
- In bacteria such as E. coli, **polymerase III** is the main replication enzyme, while polymerase I, II, IV and V are responsible for error checking and repair.

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- In eukaryotic cells, polymerases alpha, delta, and epsilon are the primary polymerases involved in DNA replication.
- Because replication proceeds in the 5' to 3' direction on the leading strand, the newly formed strand is **continuous**.

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- The **lagging strand** begins replication by binding with multiple primers.
- Each primer is only several bases apart.
- DNA polymerase then adds pieces of DNA, called **Okazaki fragments**, to the strand between primers.
- This process of replication is discontinuous as the newly created fragments are disjointed.

Termination

- Once both the continuous and discontinuous strands are formed, an enzyme called **exonuclease** removes all RNA primers from the original strands.
- These primers are then replaced with appropriate bases.
- Another exonuclease "proofreads" the newly formed DNA to check, remove and replace any errors.

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- Another enzyme called **DNA ligase** joins **Okazaki fragments** together forming a single unified strand.
- The ends of the linear DNA present a problem as DNA polymerase can only add nucleotides in the 5′ to 3′ direction.
- The ends of the parent strands consist of repeated DNA sequences called telomeres.

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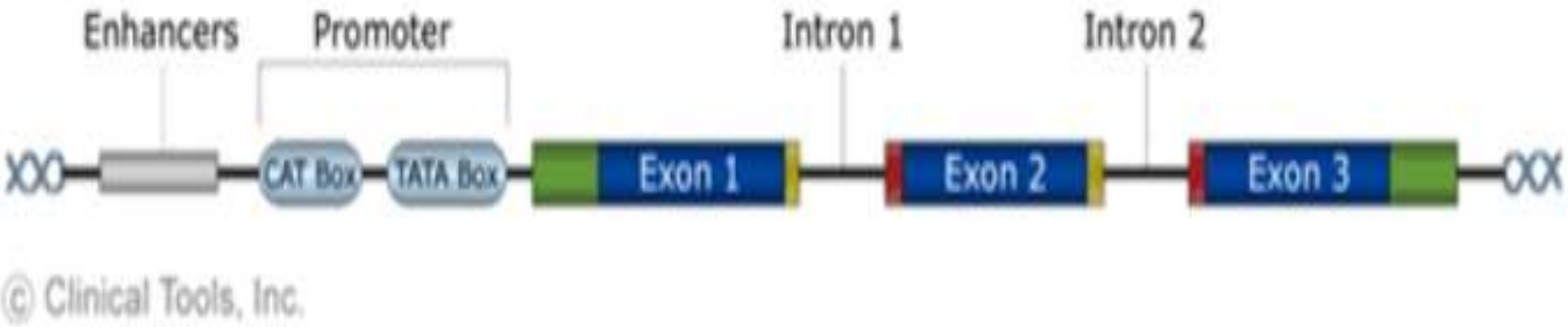
- Telomeres act as protective caps at the end of chromosomes to prevent nearby chromosomes from fusing.
- A special type of DNA polymerase enzyme called **telomerase** catalyzes the synthesis of telomere sequences at the ends of the DNA.
- Once completed, the parent strand and its complementary DNA strand coils into the familiar double helix shape.
- In the end, replication produces two DNA molecules, each with one strand from the **parent molecule** and **one new strand** hence the name semi-conservative

DNA transcription



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- Exons code for amino acids and collectively determine the amino acid sequence of the protein product. It is these portions of the gene that are represented in final mature mRNA molecule.
- Introns are portions of the gene that do not code for amino acids, and are removed (spliced) from the mRNA molecule before translation.

Transcription

- Transcription is the process of RNA synthesis, controlled by the interaction of promoters and enhancers.
- Several different types of RNA are produced, including **messenger RNA**(**mRNA**), which specifies the sequence of amino acids in the protein product, plus **transfer RNA** (**tRNA**) and **ribosomal RNA** (**rRNA**), which play a role in the translation process.

Gene control regions

- **Start site**. A start site for transcription.
- **A promoter**. A region a few hundred nucleotides 'upstream' of the gene (toward the 5' end). It is not transcribed into mRNA, but plays a role in controlling the transcription of the gene. Transcription factors bind to specific nucleotide sequences in the promoter region and assist in the binding of RNA polymerases.
- **Enhancers**. Some transcription factors (called activators) bind to regions called 'enhancers' that increase the rate of transcription. These sites may be thousands of nucleotides from the coding sequences or within an intron. Some enhancers are conditional and only work in the presence of other factors as well as transcription factors.
- **Silencers**. Some transcription factors (called repressors) bind to regions called 'silencers' that depress the rate of transcription

Transcription involves four steps:

Initiation.

- With help of RNA polymerase, the DNA molecule unwinds and separates to form a small **open complex** .
- RNA polymerase binds to the promoter of the **template strand**.

Elongation.

- RNA polymerase moves along the template strand, synthesising an mRNA molecule from the template/antisense strand.
- Unlike replication, RNA polymerase zips DNA back up as it goes keeping only 10-20 bases at a time exposed at a time
- In prokaryotes RNA polymerase is a holo-enzyme consisting of a number of subunits, including a **sigma factor** (transcription factor) that recognizes the promoter.
- In eukaryotes there are three RNA polymerases: I, II and III. The process includes a proofreading mechanism.

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Termination.

- In prokaryotes there are two ways in which transcription is terminated. In **Rho-dependent termination**, a protein factor called "Rho" is responsible for disrupting the complex involving the template strand, RNA polymerase and RNA molecule.
- In **Rho-independent termination**, a loop forms at the end of the RNA molecule, causing it to detach itself.
- Termination in eukaryotes is more complicated, involving the addition of additional adenine nucleotides at the 3' of the RNA transcript (a process referred to as **polyadenylation**).
- Once the RNA polymerase reaches the edge of a gene, termination occurs. The enzyme detaches from the gene and the DNA is returned to its original state

Processing.

- After transcription the immature **mRNA molecule** is processed in a number of ways: introns are removed and the exons are spliced together to form a mature mRNA molecule consisting of a single protein-coding sequence.
- RNA synthesis involves the normal base pairing rules, but the base thymine is replaced with the base **uracil** and it is single stranded

Translation

- In translation the mature mRNA molecule is used as a template to assemble a series of amino acids to produce a polypeptide with a specific amino acid sequence.
- The complex in the cytoplasm at which this occurs is called a **ribosome**.
- Ribosomes are a mixture of ribosomal proteins and ribosomal RNA (rRNA), and consist of a **large subunit** and a **small subunit**

Translation

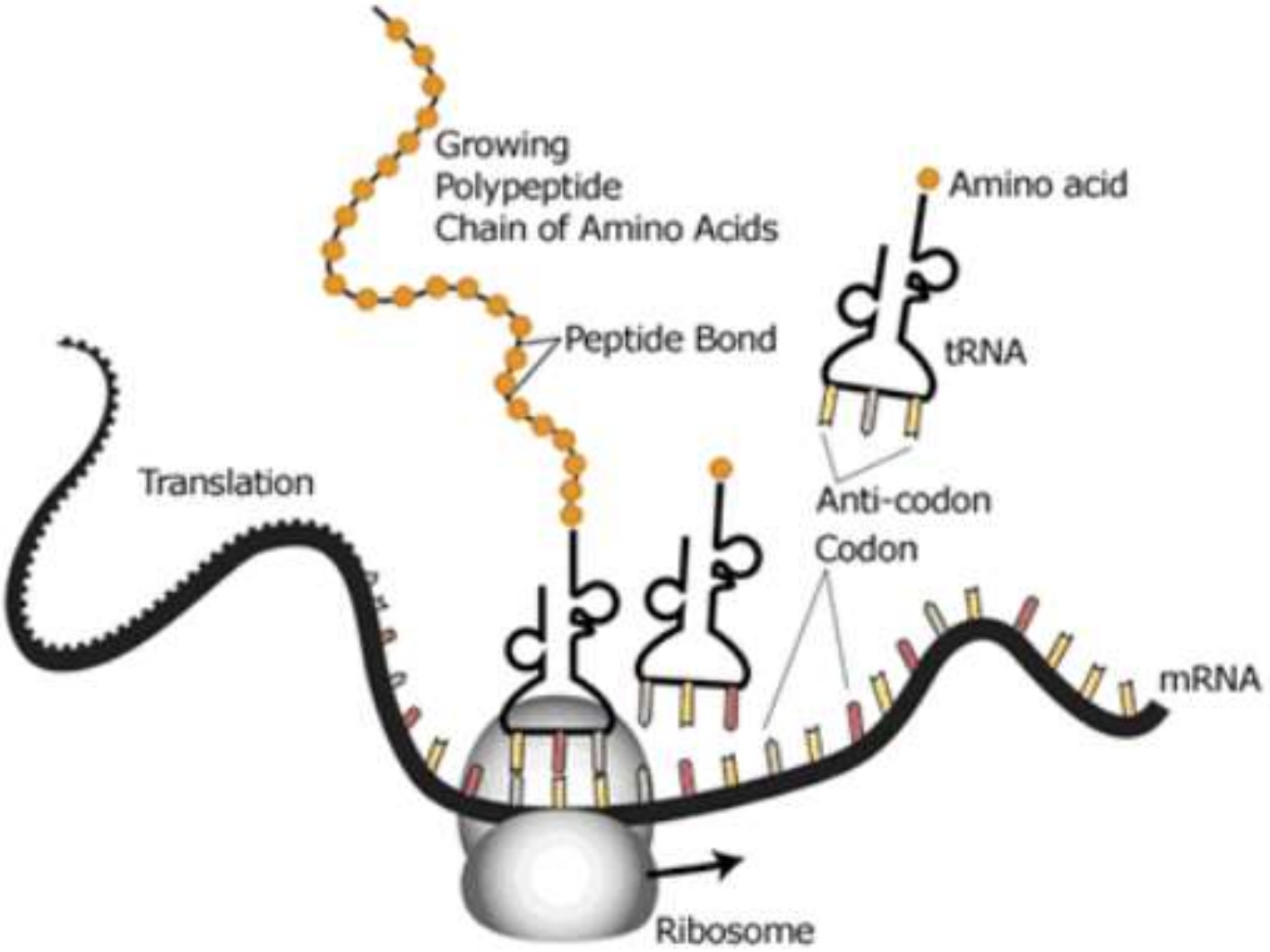

Image adapted from: National Human Genome Research Institute.

Translation involves four steps:

Initiation.

- The small subunit of the ribosome binds at the 5' end of the mRNA molecule and moves in a 3' direction until it meets a **start codon** AUG (methionine). It then forms a complex with the large unit of the ribosome complex and an initiation tRNA molecule.
- Each set of 3 bases on a mRNA is called a codon and codes for a specific anti-codon which will be carried by a **specific tRNA** and each tRNA is covariantly linked to a particular amino acid.

Elongation.

- Subsequent codons on the mRNA molecule determine which tRNA molecule linked to an amino acid binds to the mRNA.
- An enzyme peptidyl transferase links the amino acids together using peptide bonds. The process continues, producing a chain of amino acids as the ribosome moves along the mRNA molecule.

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Termination.

- Translation is terminated when the ribosomal complex reaches one or more stop codons (UAA, UAG, UGA).
- The ribosomal complex in eukaryotes is larger and more complicated than in prokaryotes.

Post-translation processing of the protein

- Protein folding, further modification and packaging in organelles like ER and Golgi apparatus resp.

Gene Regulation

- In addition, the processes of transcription and translation are divided in eukaryotes between the nucleus (transcription) and the cytoplasm (translation), which provides more opportunities for the regulation of gene expression.
- Gene regulation is a label for the cellular processes that control the rate and manner of gene expression.
- A complex set of interactions between genes, RNA molecules, proteins (including transcription factors) and other components of the expression system determine when and where specific genes are activated and the amount of protein or RNA product produced.

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- ✓ Some genes are expressed continuously, as they produce proteins involved in basic metabolic functions
- ✓ some genes are expressed as part of the process of cell differentiation and
- ✓ some genes are expressed as a result of cell differentiation.

Mechanisms of gene regulation include:

- Regulating the rate of transcription. This is the most economical method of regulation.
- Regulating the processing of RNA molecules, including alternative splicing to produce more than one protein product from a single gene.
- Regulating the stability of mRNA molecules.
- Regulating the rate of translation

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- **Transcription factors** are proteins that play a role in regulating the transcription of genes by binding to specific regulatory nucleotide sequences.