Molecular biology (2)

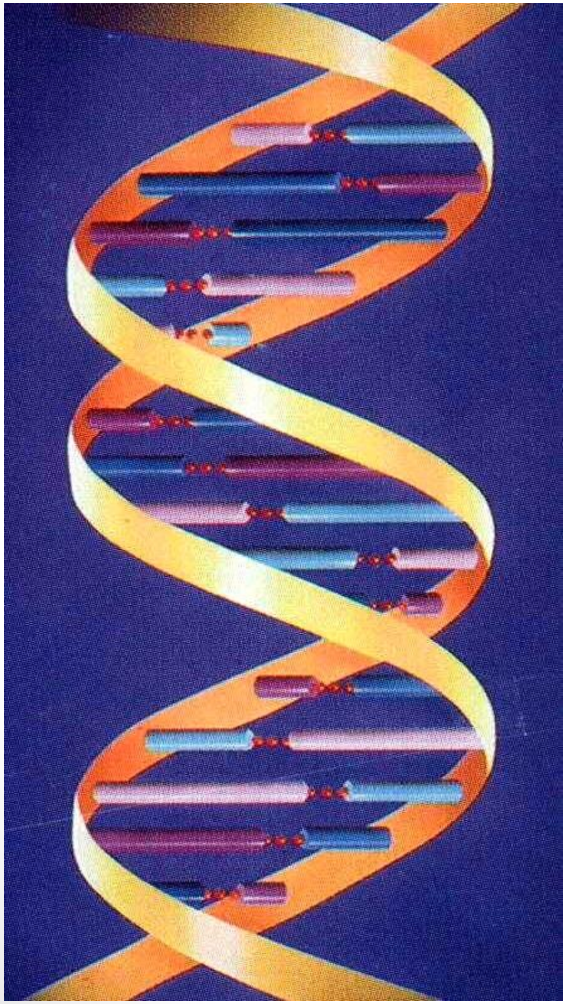
(Foundation Block)

Objectives

By the end of this lecture, the students should be able to:

- To understand DNA replication
- To know the transcription of genetic material into messenger RNA
- To get an idea about the translation of mRNA into a functional protein.

DNA is the genetic material



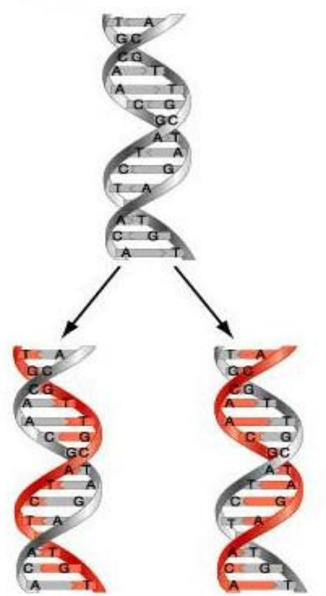
Therefore it must:

1. Replicate faithfully.
2. Have the coding ability to produce proteins for all cellular functions.

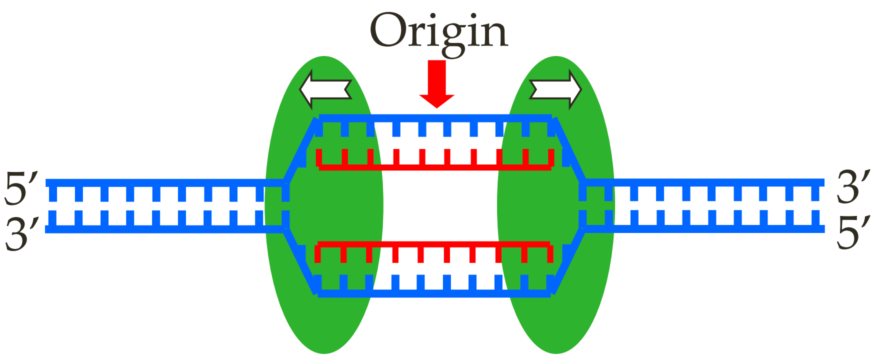
Features of Eukaryotic DNA Replication

① Semiconservative with respect to parental strand:

- Daughter DNA molecules contain one **parental strand** and one **newly-replicated strand**.

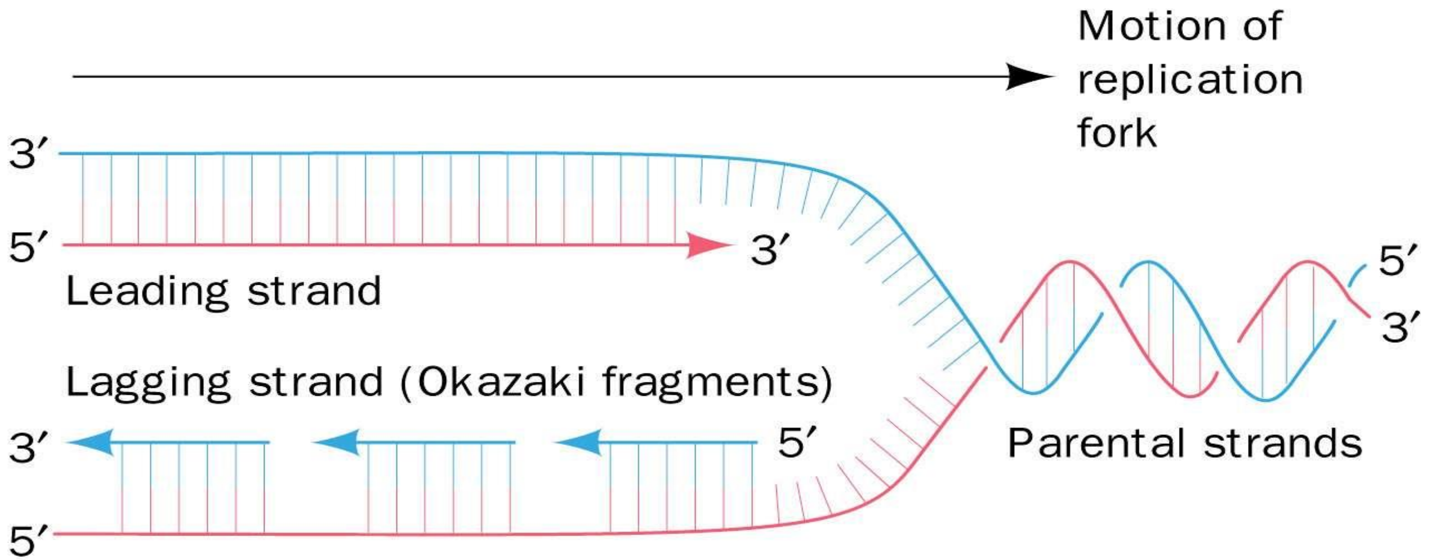


② Bidirectional with multiple origins of replication.



③ Primed by short stretches of RNA.

④ Semi-discontinous

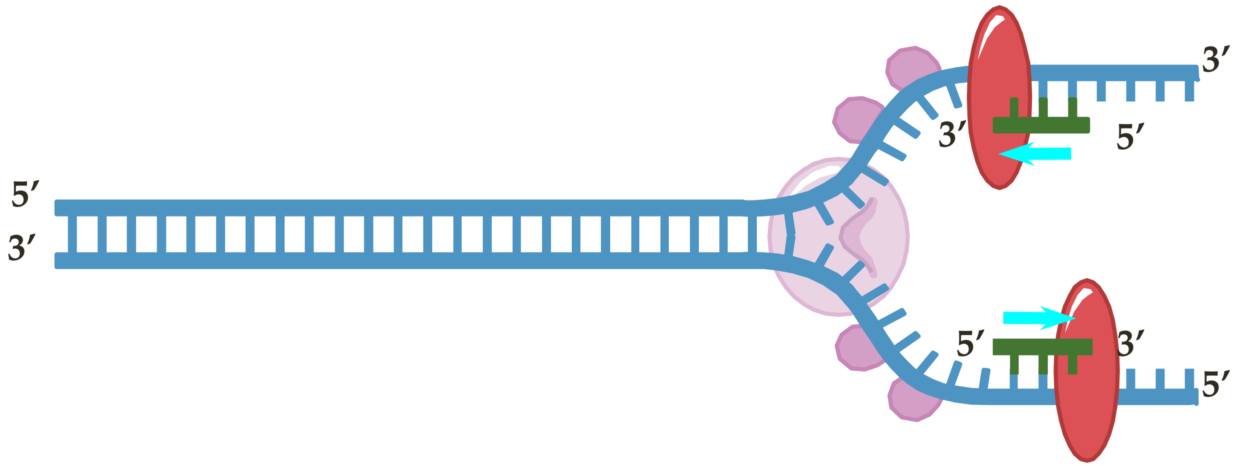


Semidiscontinuous DNA replication. In DNA replication, both daughter strands (*leading strand red*, *lagging strand blue*) are synthesized in their 5’ → 3’ directions

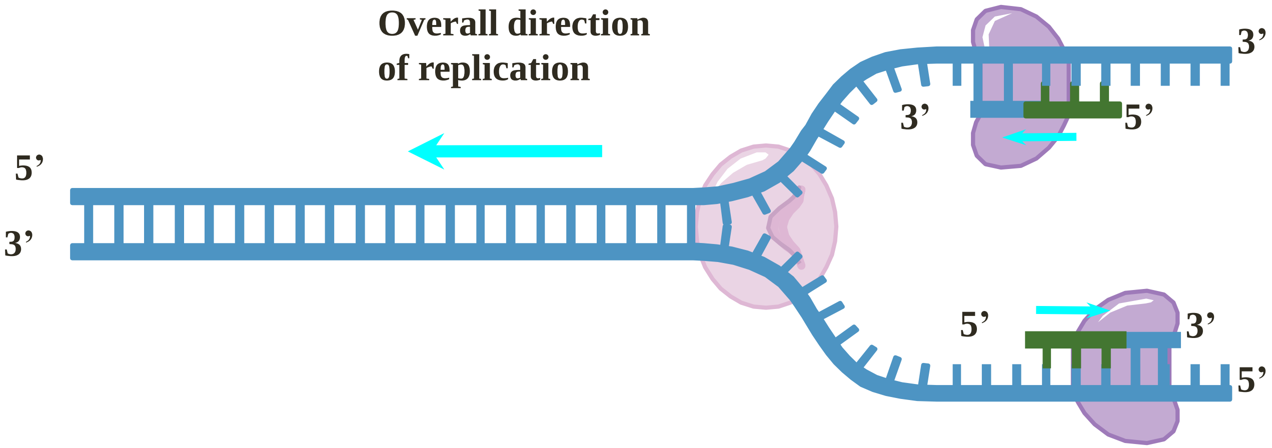
Proteins involved in DNA Replication

1. DNA Helicase.
2. Single-stranded DNA binding proteins.
3. DNA Primase.
4. DNA polymerases (5 types: α; β; γ; δ; ε).
5. DNA ligase.
6. Topoisomerases:
    1. Topoisomerase I.
    2. Topoisomerase II.
7. Telomerases

Steps in DNA Replication

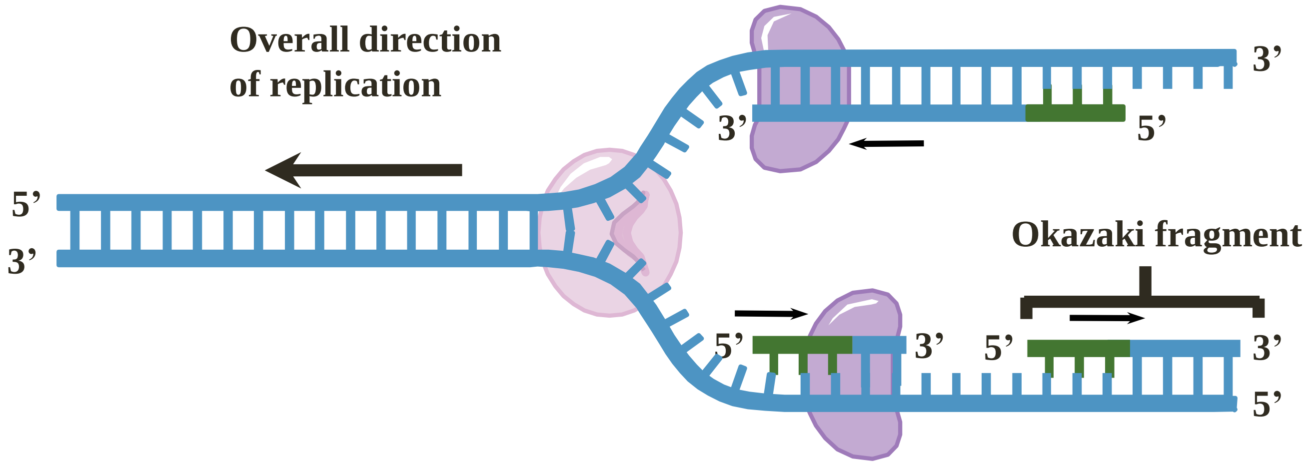


- **Helicase protein** binds to DNA sequences called origins and unwinds DNA strands.
- **Single-Stranded binding proteins** prevent single strands from rewinding.
- **Primase protein** makes a short segment of **RNA primer** complementary to the DNA.

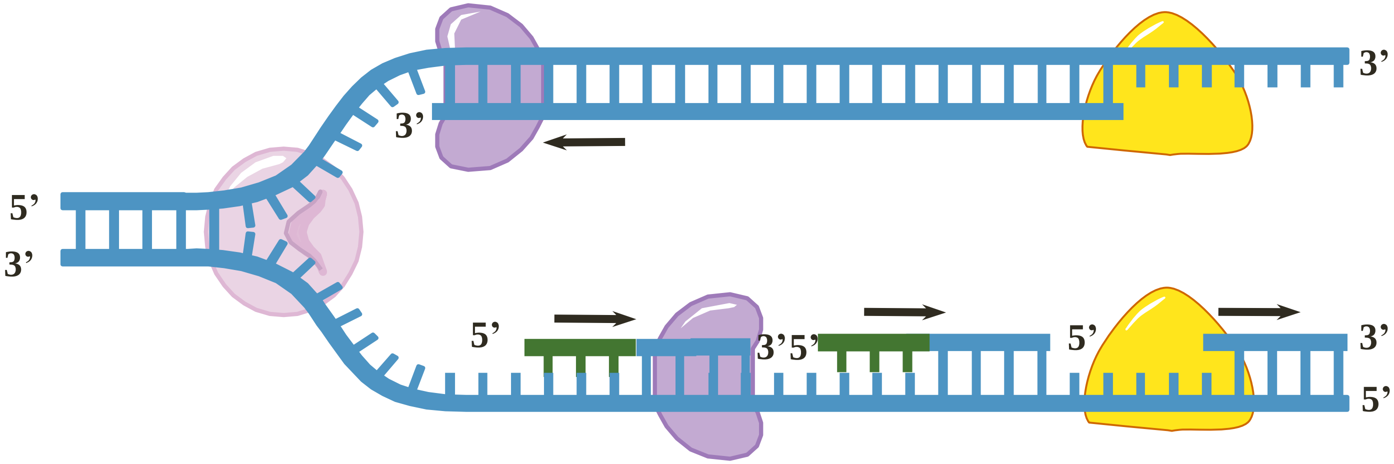


DNA polymerase:

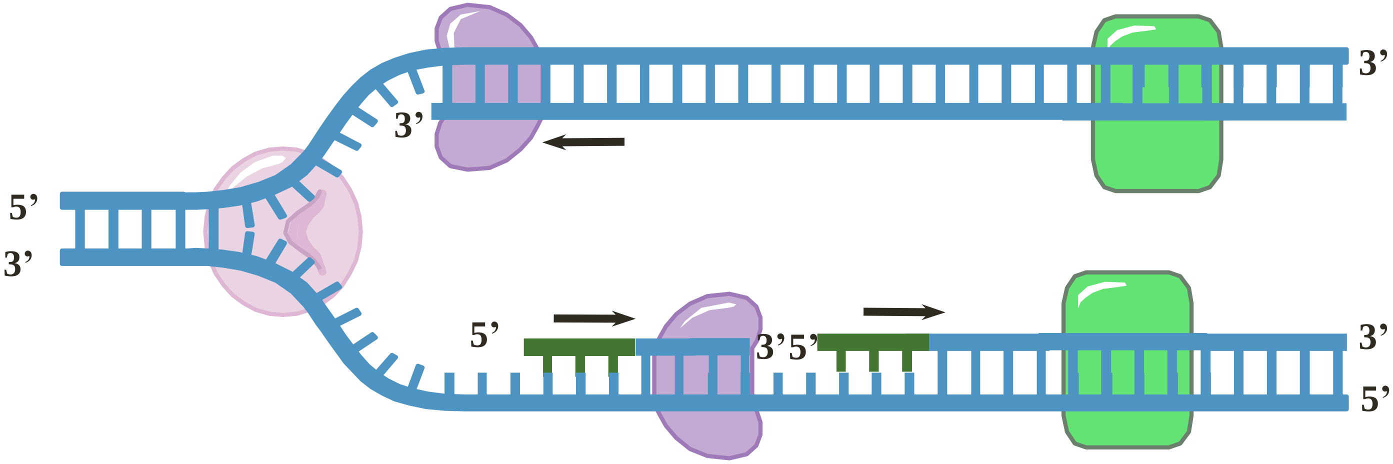
- Adds DNA nucleotides to the RNA primer.
- Proofreads bases added and replaces incorrect nucleotides



- Leading strand synthesis continues in a 5’ to 3’ direction.
- Discontinuous synthesis produces 5’ to 3’ DNA segments (Okazaki fragments).

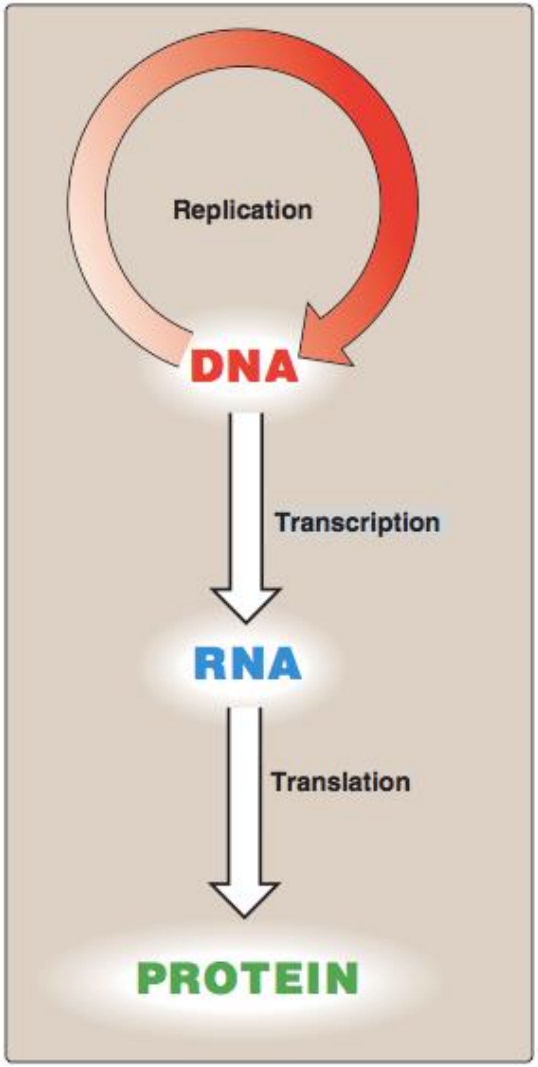


Exonuclease activity of DNA polymerase removes RNA primers



- DNA polymerase fills the gaps.
- **Ligase** forms bonds between sugar-phosphate backbone

The central dogma of Molecular Biology



*A portion of **DNA**, called a **gene**, is transcribed into **RNA**.*

*RNA is translated into **proteins**.*

Transcription (mRNA synthesis)

- A portion of **DNA** (a **gene**) is transcribed into messenger RNA (**mRNA**).

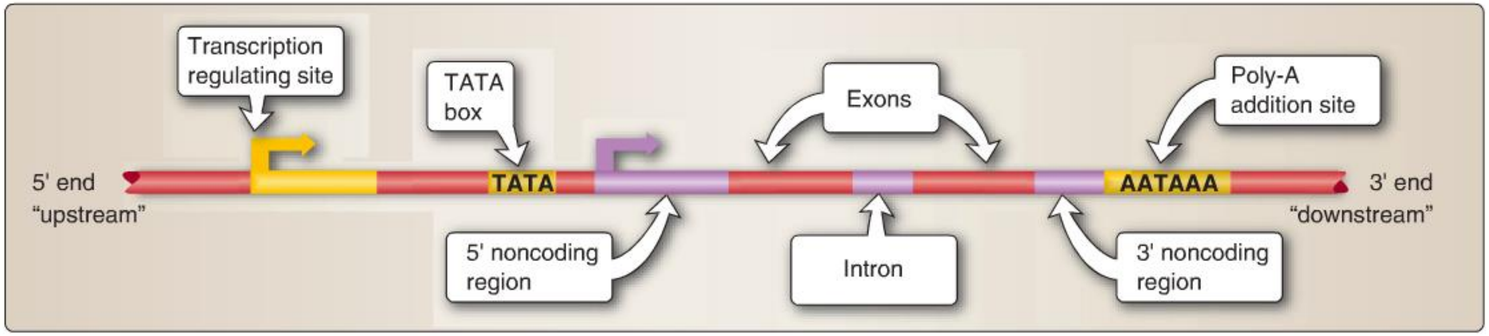
- Only one of the DNA strands is transcribed (**antisense strand**).

- The **RNA polymerase II** is responsible for this process.

- The direction of transcription is **5’ → 3’**.

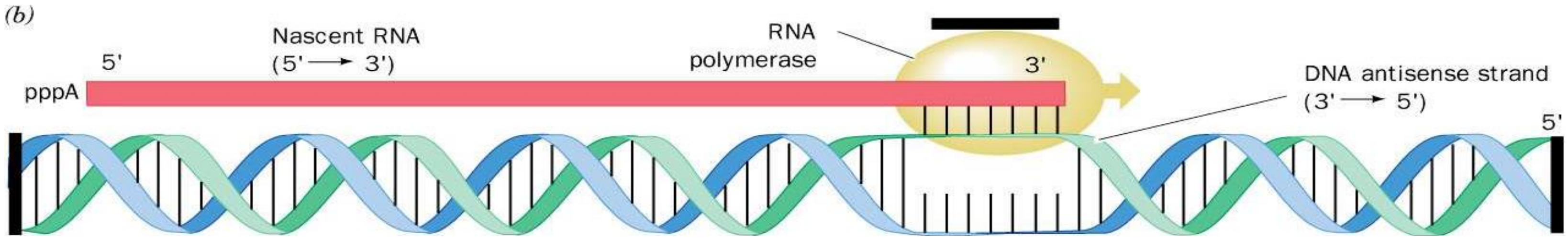
Steps of mRNA synthesis

- **Chain initiation:**
  - RNA polymerase II binds to ***promoter region*** of DNA to start transcription.



Steps of mRNA synthesis

- **Chain elongation:**
  - A portion of DNA template unwinds (opens) at the point of RNA synthesis.
  - This forms a short length of RNA-DNA hybrid.



- **Chain termination:**
  - DNA contains specific sites which stop transcription (at a sequence of 4-10 AT base pairs).

Post-transcriptional modification

- **Capping:** Addition of a methylated guanine nucleotide at 5’ end of mRNA

  <u>*Function*</u>*:*

  - *To prevent mRNA degradation by exonucleases.*
  - *It helps the transcript bind to the ribosome during protein synthesis.*

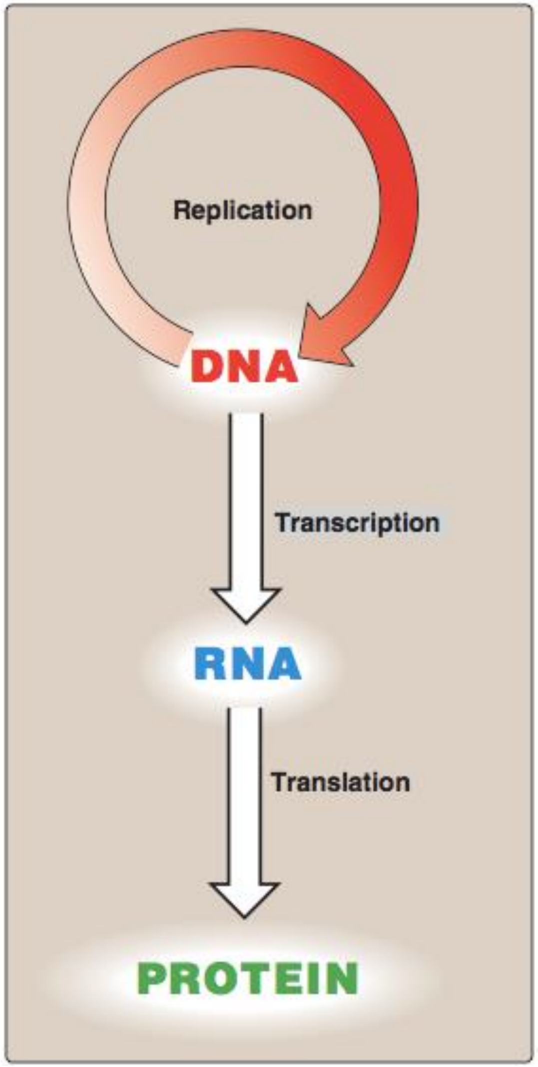
- **Polyadenylation:** Addition of a poly(A) tail (a highly conserved AAUAA sequence) at 3’ end of mRNA.

  <u>*Functions*</u>*:*

  - *To protect the mRNA from degradation*
  - *For ribosomal RNA recognition*

- **Intron removal for releasing mature mRNA from nucleus.**

The central dogma of Molecular Biology



*A portion of **DNA**, called a **gene**, is transcribed into **RNA**.*

*RNA is translated into **proteins**.*

Translation (Protein synthesis)

- A process of protein synthesis from mRNA
- mRNA has genetic codes for amino acids present in proteins.
- The **genetic code** is a dictionary that identifies the correspondence between a sequence of nucleotide bases and a sequence of amino acids.
- Each individual word in the code is composed of three nucleotide bases (**codons**).

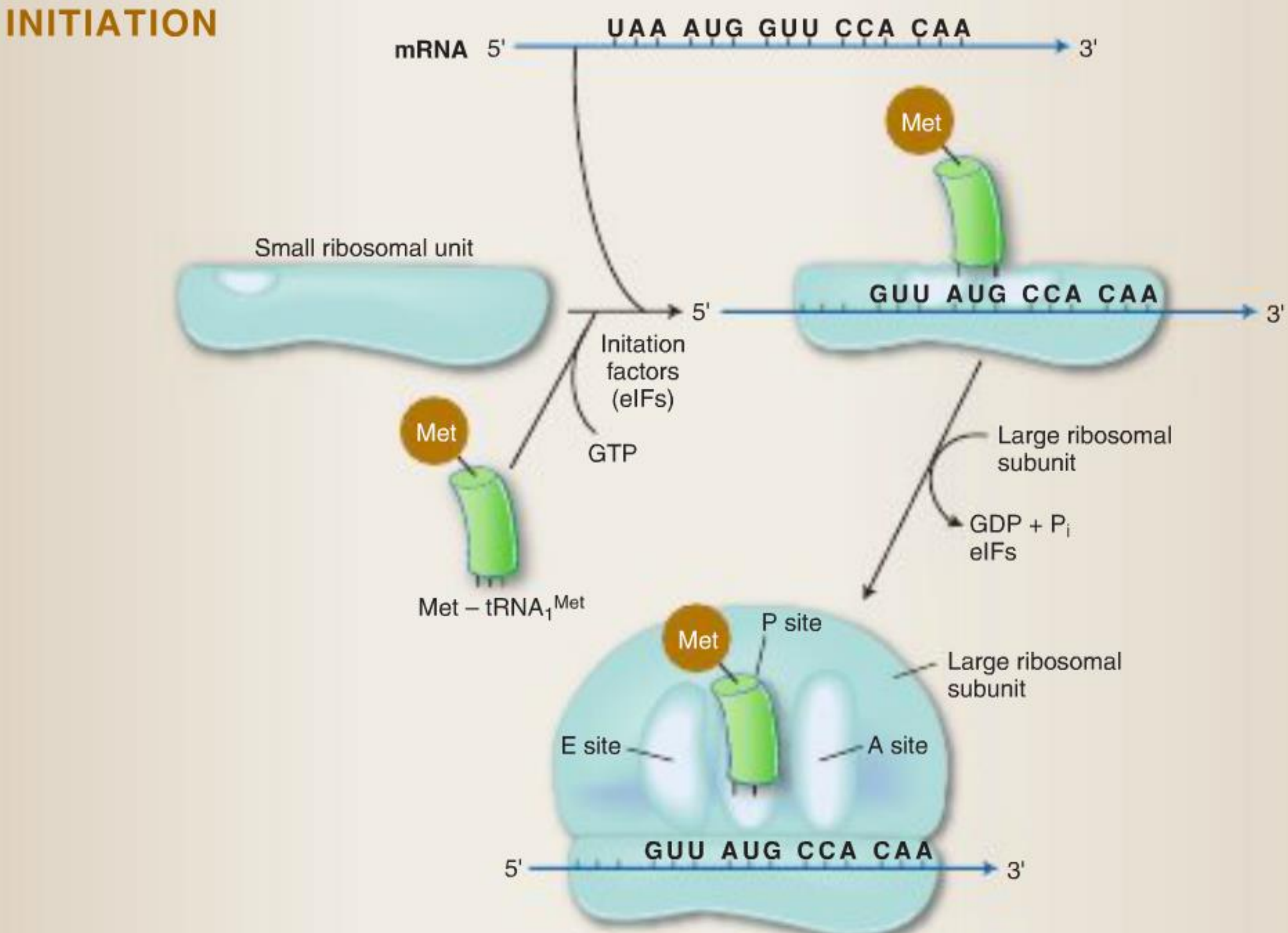
- **64 possible codons:**
  - 61 codons specify 20 amino acids
  - One start codon (AUG)
  - 3 stop codons
    UAA, UAG and UGA

First position (5′ end)	Second position				Third position (3′ end)
	U	**C**	**A**	**G**	
U	UUU UUC Phe UUA UUG Leu	UCU UCC UCA Ser UCG	UAU UAC Tyr UAA Stop UAG Stop	UGU UGC Cys UGA Stop UGG Trp	**U** **C** **A** **G**
C	CUU CUC CUA Leu CUG	CCU CCC CCA Pro CCG	CAU CAC His CAA CAG Gln	CGU CGC CGA Arg CGG	**U** **C** **A** **G**
A	AUU AUC Ile AUA AUG Met[b]	ACU ACC ACA Thr ACG	AAU AAC Asn AAA AAG Lys	AGU AGC Ser AGA AGG Arg	**U** **C** **A** **G**
G	GUU GUC GUA Val GUG	GCU GCC GCA Ala GCG	GAU GAC Asp GAA GAG Glu	GGU GGC GGA Gly GGG	**U** **C** **A** **G**

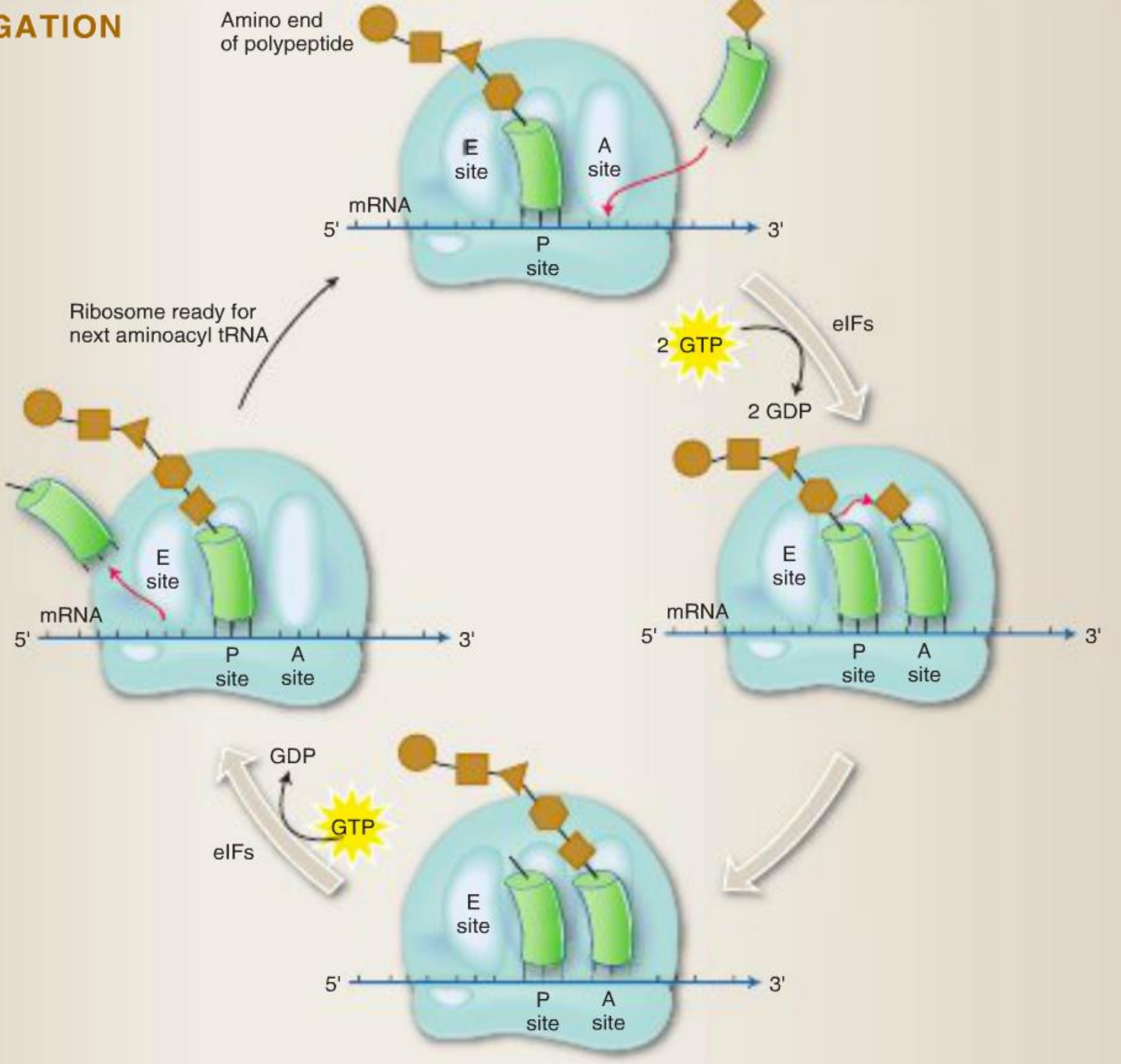
Components required for Translation

1. Amino acids.
2. Transfer RNA (tRNA).
3. Aminoacyl-tRNA synthetases.
4. mRNA.
5. Functionally competent ribosomes.
6. Protein factors.
7. ATP and GTP.

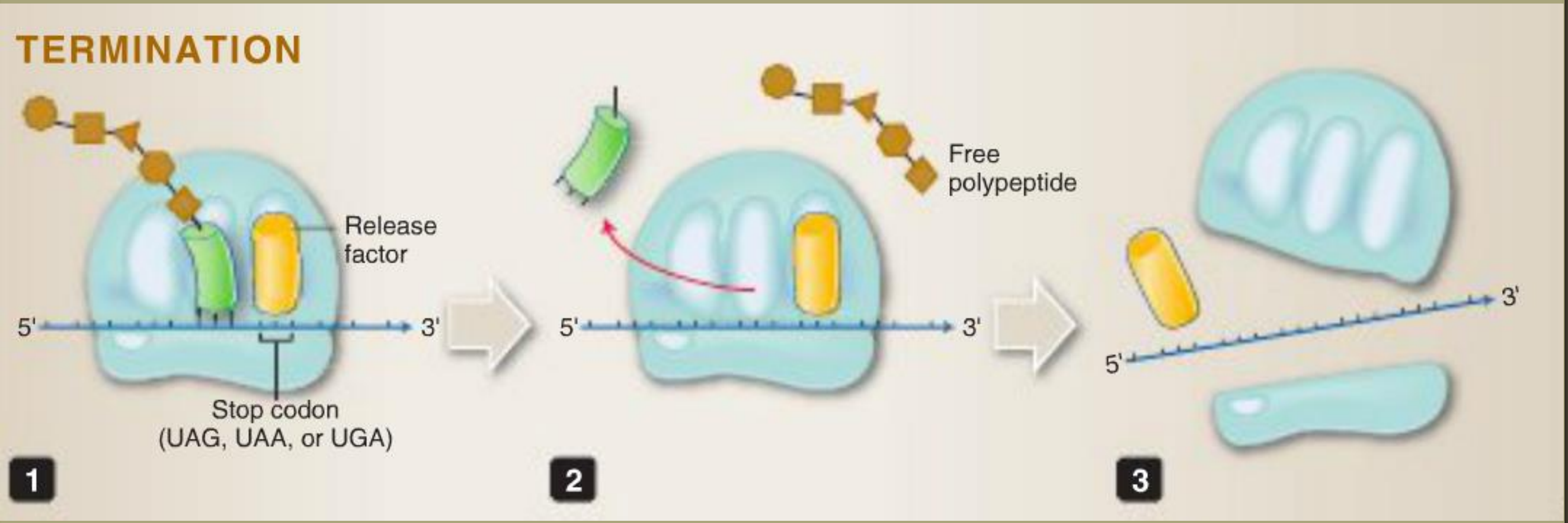
Steps in Protein Translation



ELONGATION



Amino end
of polypeptide
E
site
A
site
mRNA
5'
3'
P
site
eIFs
2 GTP
2 GDP
E
site
mRNA
5'
3'
P
site
A
site
E
site
5'
3'
P
site
A
site
GDP
GTP
eIFs
E
site
mRNA
5'
3'
P
site
A
site
Ribosome ready for
next aminoacyl tRNA


TERMINATION
Release factor
5'
3'
Stop codon (UAG, UAA, or UGA)
1
Free polypeptide
5'
3'
2
3'
5'
3

Take home messages

- DNA is the genetic material, so it must replicate faithfully and have the coding ability to produce proteins for all cellular functions.
- Only one strand of DNA (antisense strand) is transcribed into mRNA.
- The synthesized mRNA is protected from the destruction and prepared for translation through post-transcriptional modification.
- mRNA transcription and protein synthesis processes are the same in both prokaryotic eukaryotic cells with some differences.

References

Lippincott's Illustrated reviews: Biochemistry 6th edition, Unit 6, chapters 29, 30 and 31, Pages 395-448.

Lippincott's Illustrated reviews: Cell and Molecular Biology, Unit 2, Chapters 7, 8 and 9, Pages 69-106.