Molecular biology (2)

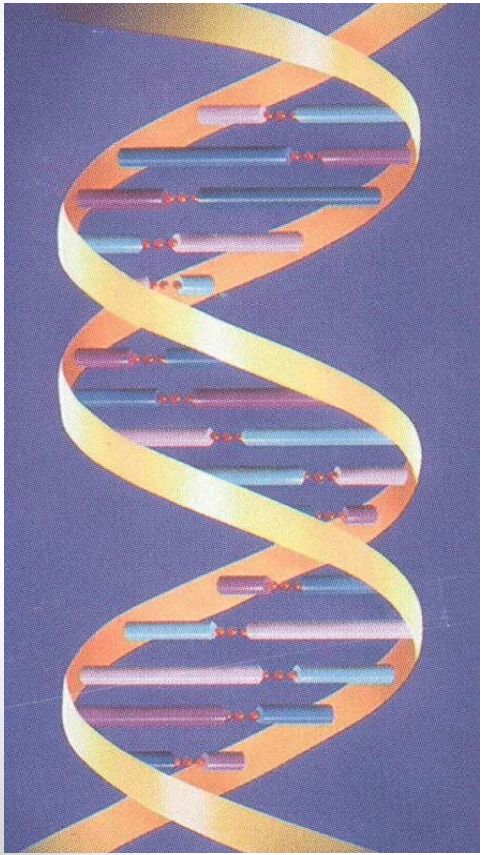
(Foundation Block)

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:

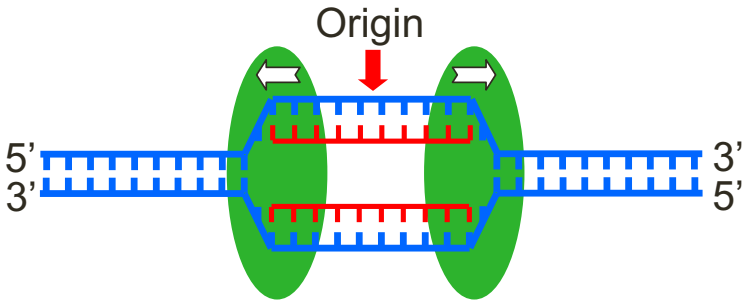
1. Replicate faithfully.
1. Have the coding ability to produce proteins for all cellular functions.

Features of Eukaryotic DNA Replication

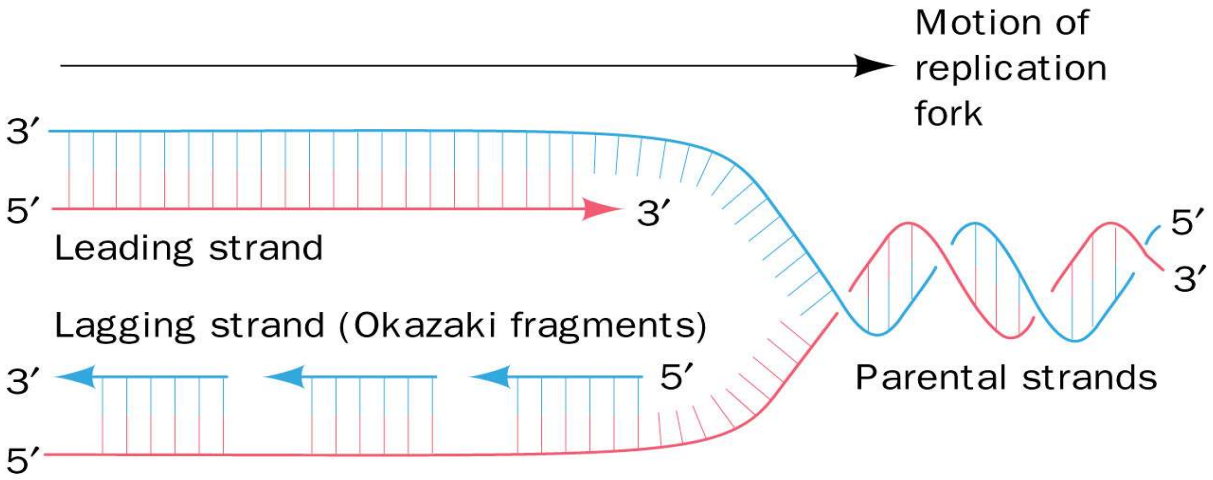
Semiconservative with respect to parental strand:

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

Bidirectional with multiple origins of replication.



Primed by short stretches of RNA.
Semi-discontinous



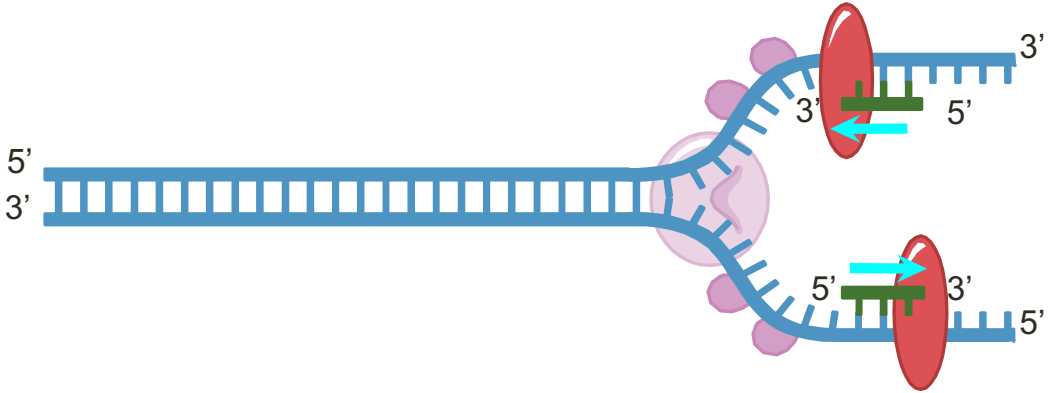
Semidiscontinuous DNA replication. In DNA replication, both daughter strands (leading strand red, lagging strand blue) are synthesized in their 5' ® 3' directions

Proteins involved in DNA Replication

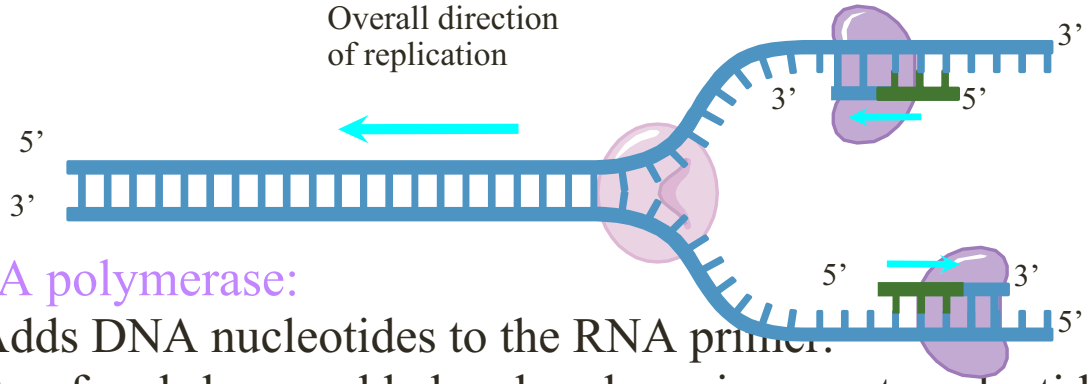
DNA Helicase.
Single-stranded DNA binding proteins.
DNA Primase.
DNA polymerases (5 types: α; β; γ; δ; ε).
DNA ligase.
Topoisomerases:
- Topoisomerase I.
- Topoisomerase II.

Telomerases

Steps in DNA Replication

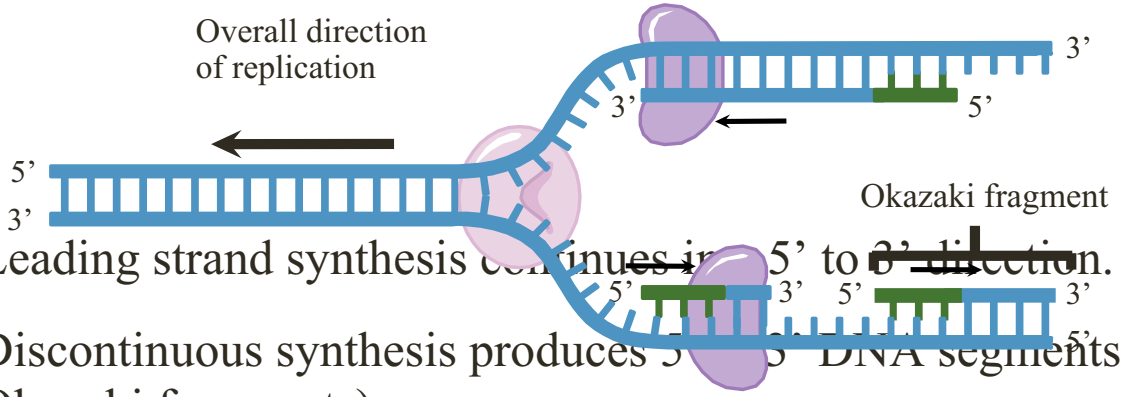


- Helicase protein binds to DNA sequences called origins and unwinds DNA strands.
- Single-Stranded binding proteins prevent single strands from rewinding.
- Primase protein makes a short segment of RNA primer complementary to the DNA.

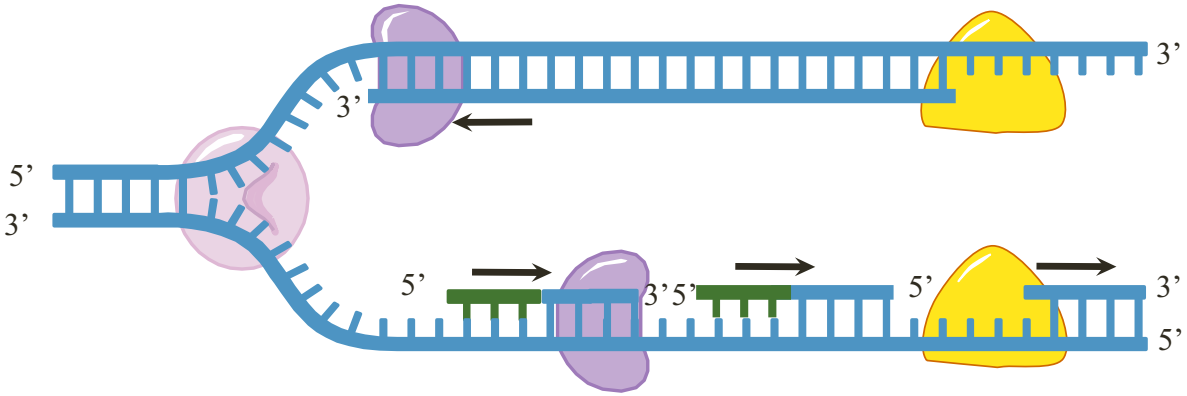


DNA polymerase:

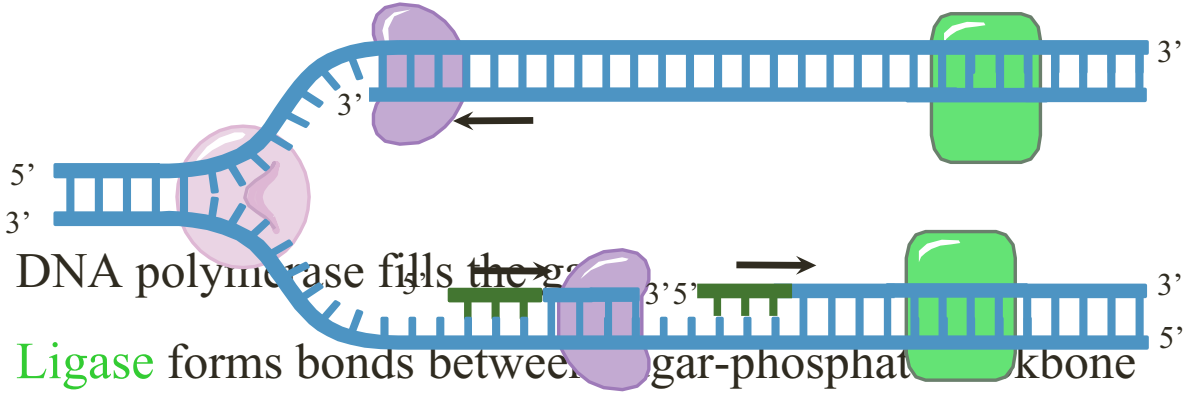
- Adds DNA nucleotides to the RNA primer.
- Proofreads bases added and replaces incorrect nucleotides



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

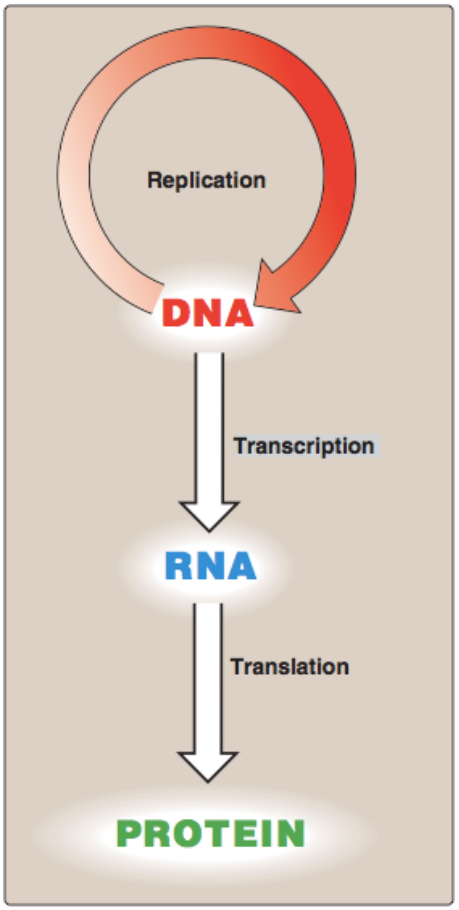


Exonuclease activity of DNA polymerase removes RNA primers



- DNA polymerase fills the ga
- Ligase forms bonds betwee gar-phosphat kbone

The central dogma of Molecular Biology



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

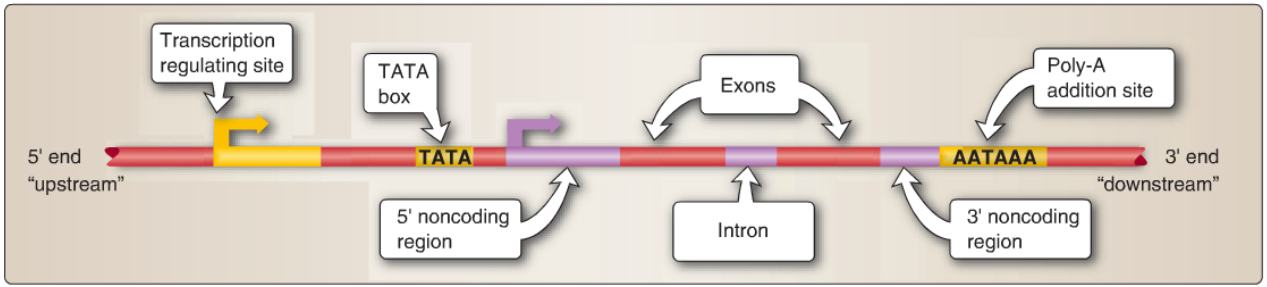
RNA is translated into proteins.

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’.

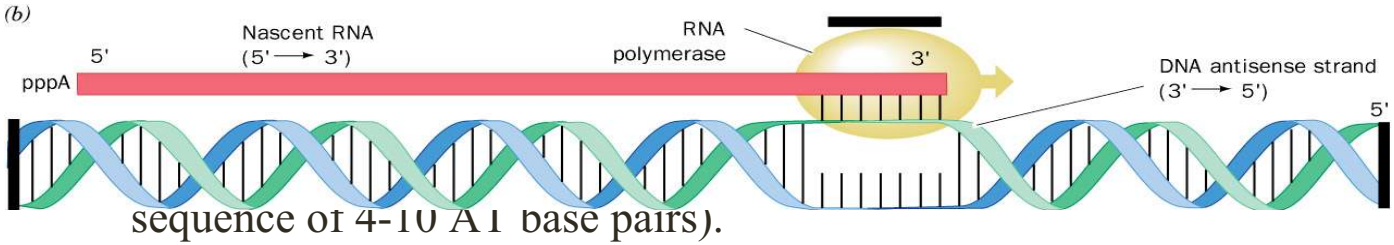
Steps of mRNA synthesis

- 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.



sequence of 4-10 A-T base pairs).

Post-transcriptional modification

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

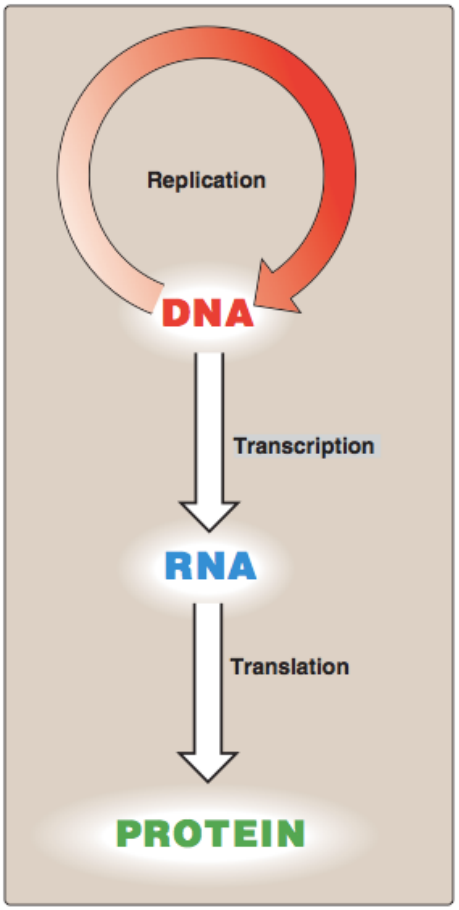
  Function:
  - To prevent mRNA degradation by exonucleases.
  - It helps the transcript bind to the ribosome during protein synthesis.

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

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

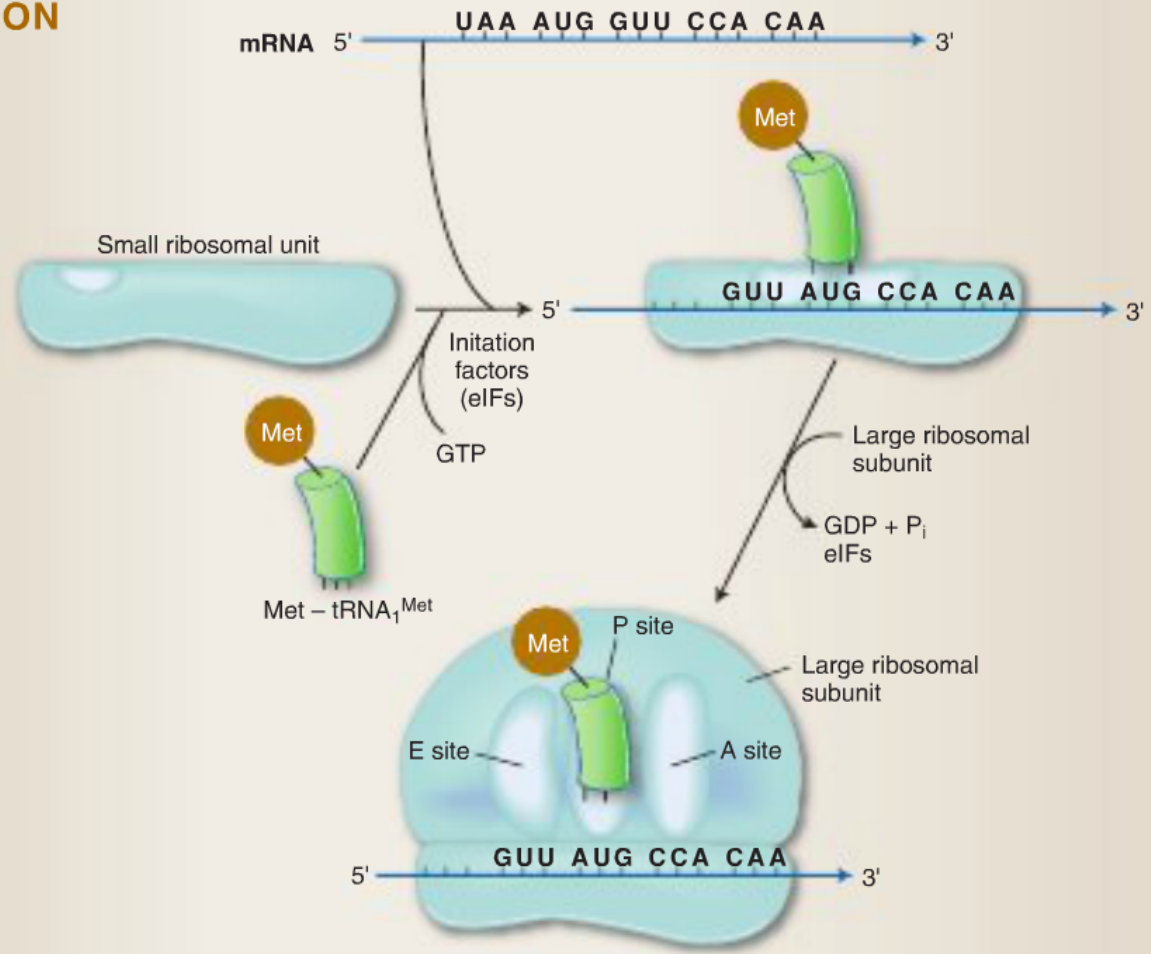
[a]Nonpolar amino acid residues are tan, basic residues are blue, acidic residues are red, and nonpolar uncharged residues are purple.
[b]AUG forms part of the initiation signal as well as coding for internal Met residues.

Components required for Translation

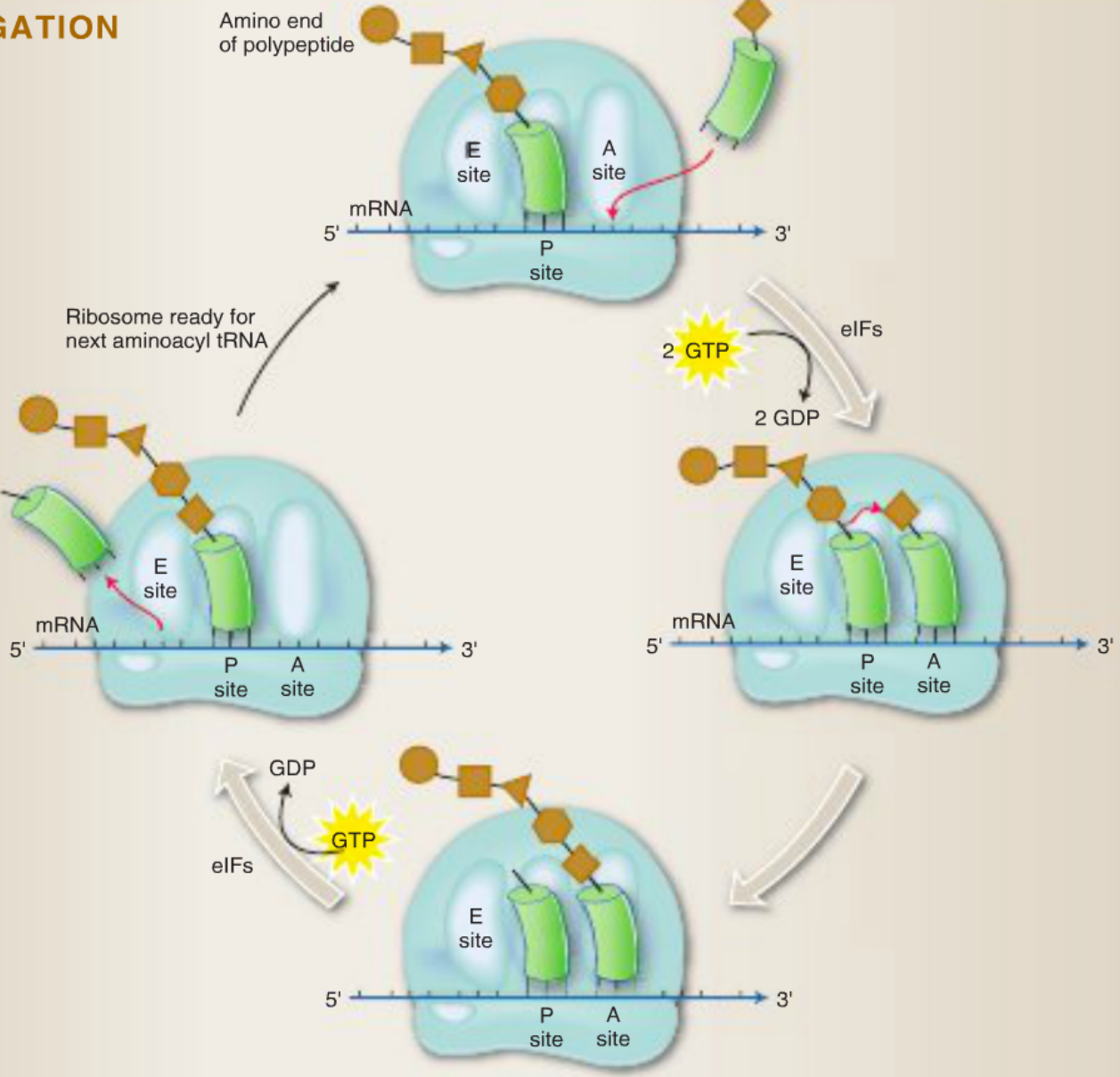
Amino acids.
Transfer RNA (tRNA).
Aminoacyl-tRNA synthetases.
mRNA.
Functionally competent ribosomes.
Protein factors.
ATP and GTP.

Steps in Protein Translation

INITIATION



ELONGATION


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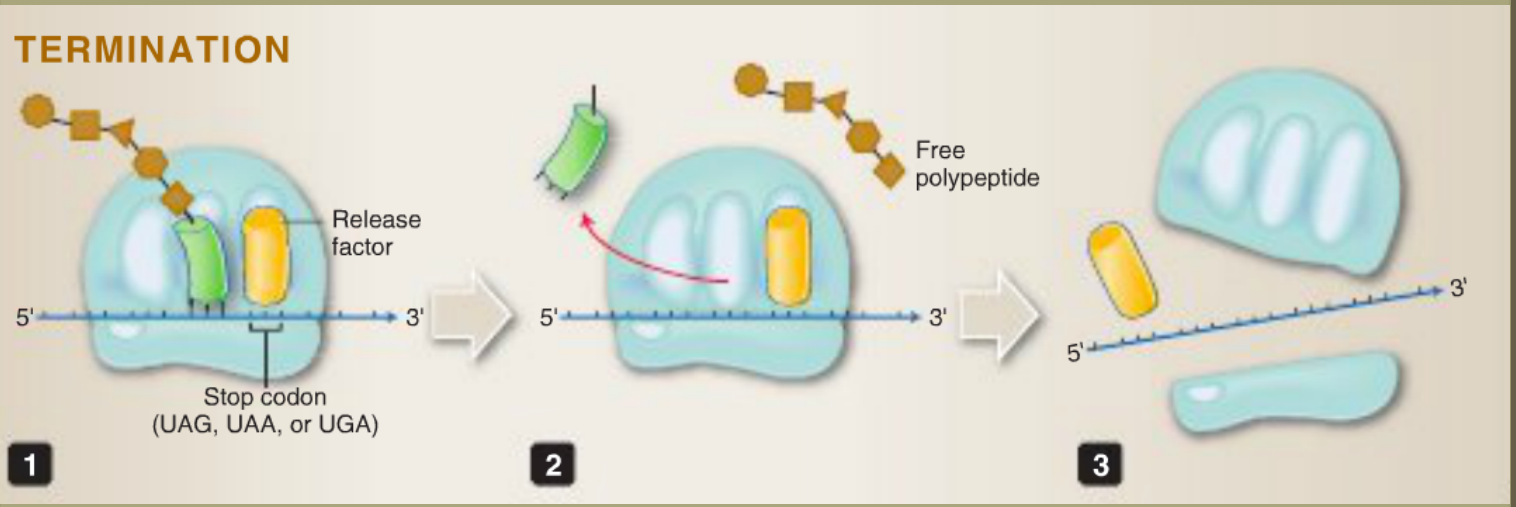
5'
3'
P
site
A
site


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