Cell and molecular Biology/ Theory

8- The Genome

DNA replication

Gene expression (Transcription and Translation)

Asst. Prof. Dr. Bushra T. Mohammed

BVM&S, MSc, PhD in Molecular Biology

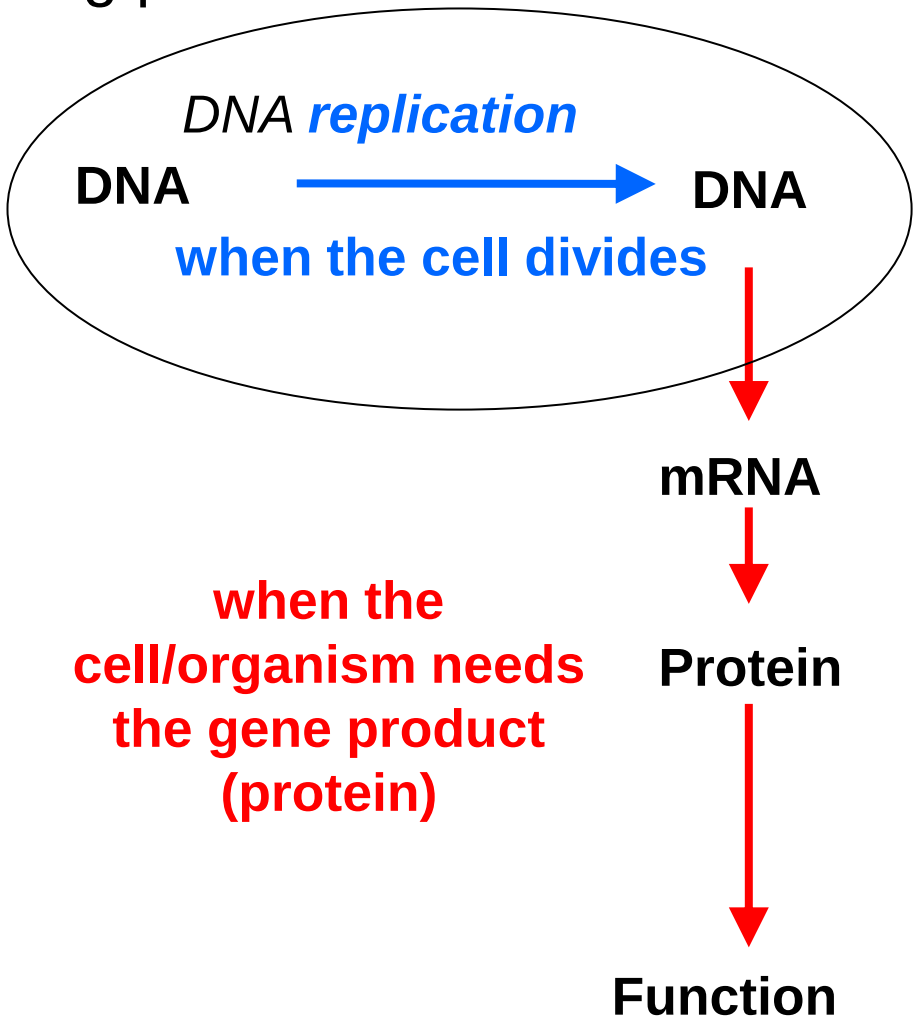
THE UNIVERSITY of DUHOK

COLLEGE OF VETERINARY MEDICINE

Learning objectives

- Understand how the genetic information transfer (Central Dogma)
- Understand how the process of DNA replication
- Explain how RNA synthesis is performed in eukaryotes.
- Explain the way that proteins are synthesized and targeted in a cell.

The central dogma describes the transfer of sequence information during DNA replication, transcription into RNA, and translation into amino-acid chains forming proteins.



Information transfer

transcription

RNA processing – splicing, capping, polyadenylation
transport to ribosomes

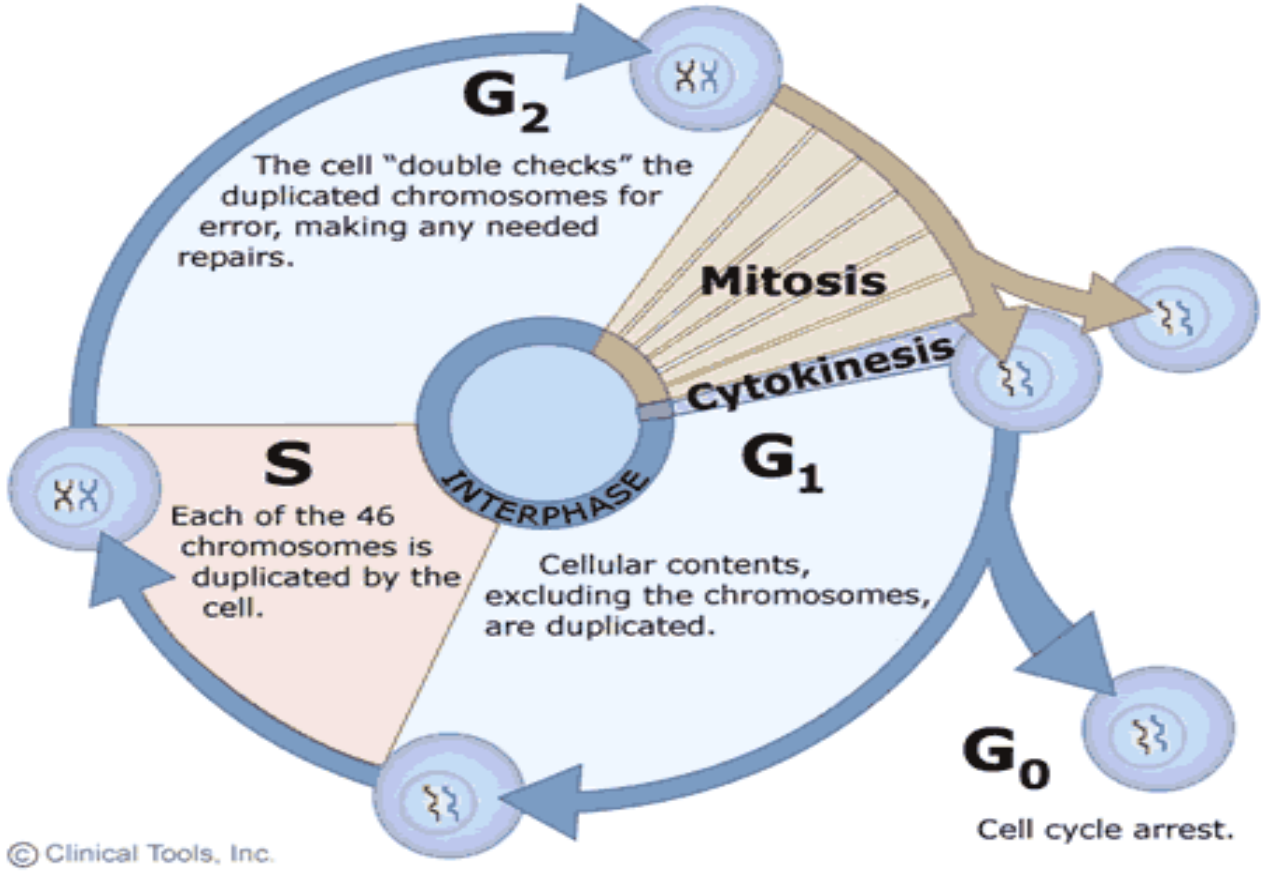
translation

post-translational modification – phosphorylation, glycosylation etc

transport to site of function

DNA replication

Deoxyribonucleic acid (DNA) contains all the information necessary for the development and function of all organisms. The replication or copy **DNA** from DNA occurs during the S or synthesis phase of the cell cycle.



Cell cycle

DNA Replication Enzymes (proteins)

Gyrase (A topisomerase): Acts to reduce torsion or twisting in the DNA double helix

DNA Helicase: Breaking hydrogen bonds to unwinds the double strand DNA

Single Strand Binding Proteins: Binds to nucleotide of DNA template to prevent premature annealing.

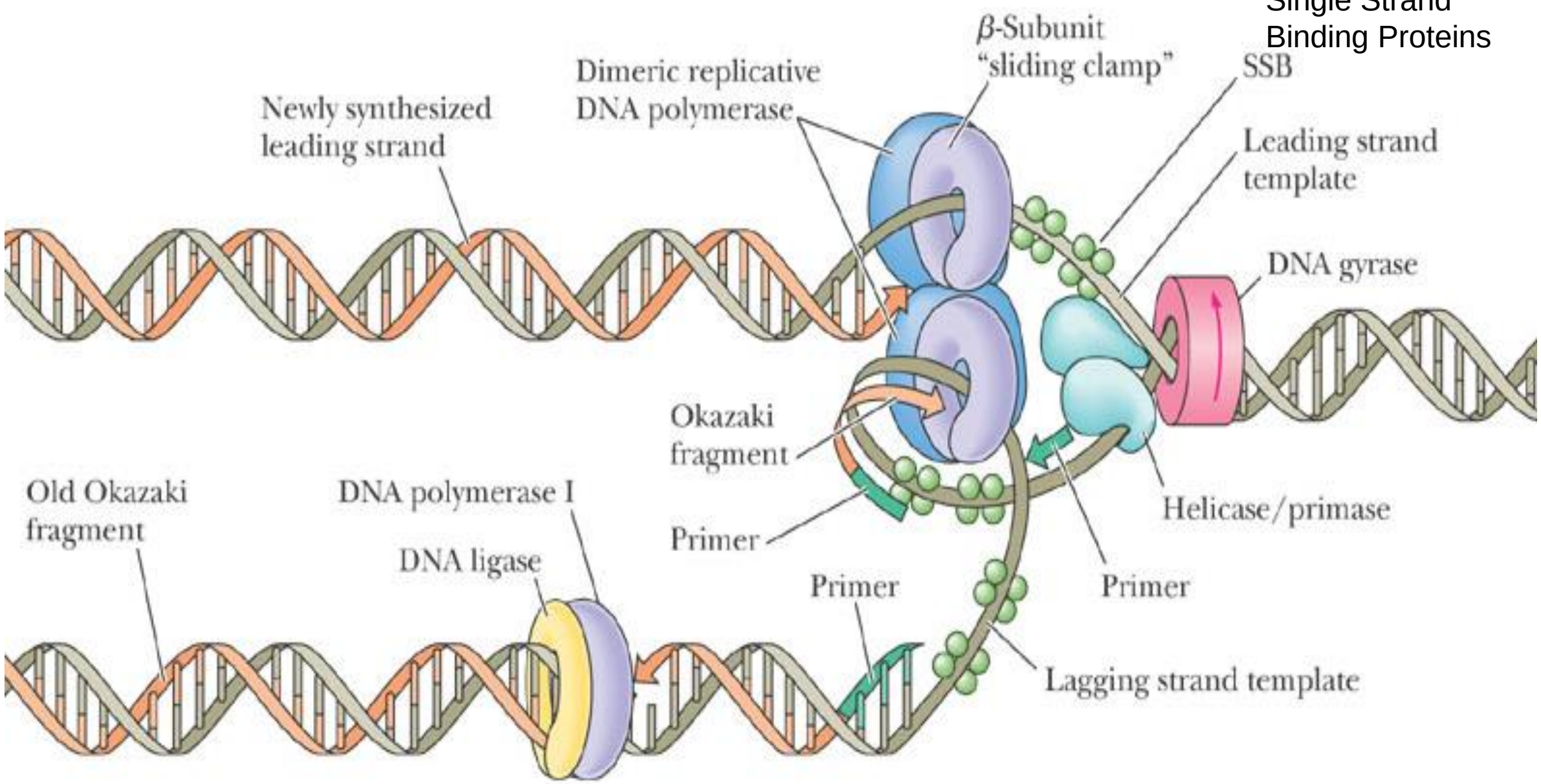
DNA Primase: Synthesis of primer to start copying

DNA Polymerase: Stimulates the formation of new DNA strands by adding a nucleotide to a free 3 end and therefore synthesizes DNA in the 5 to 3 direction.

DNA Ligase: Joins adjacent short fragments of DNA strands together

Telomerase: Helps to maintain the telomere (the end of a chromosome)

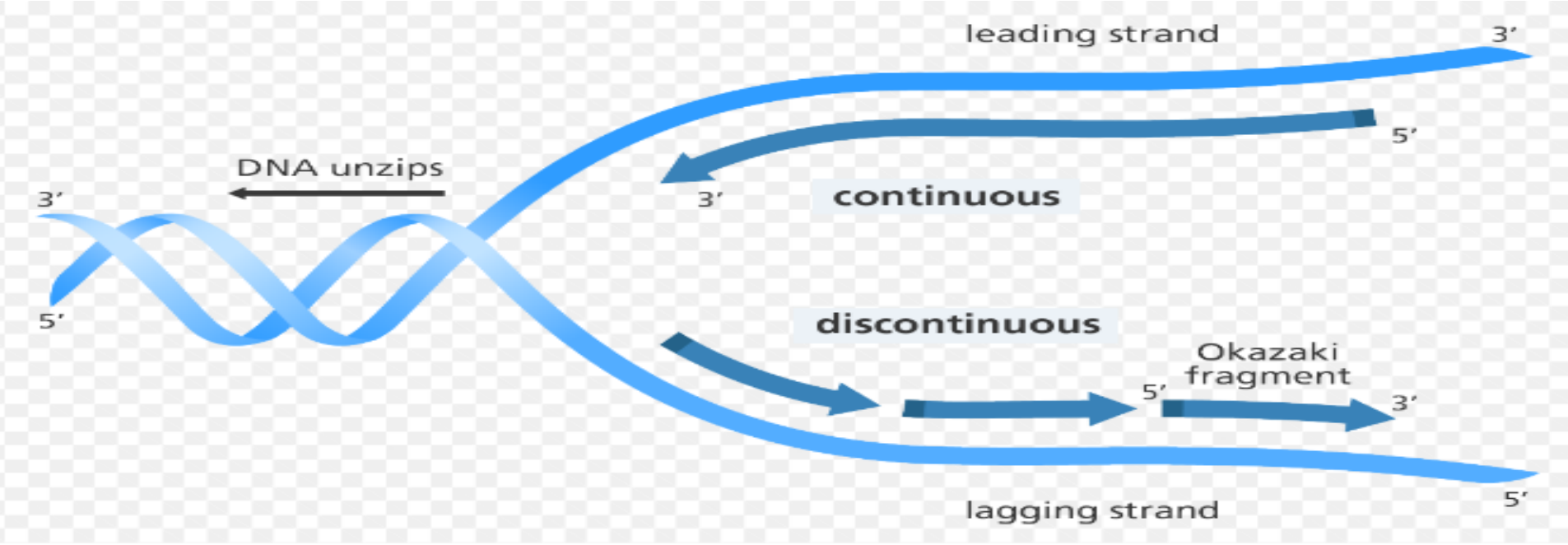
DNA replication



Steps of DNA replication

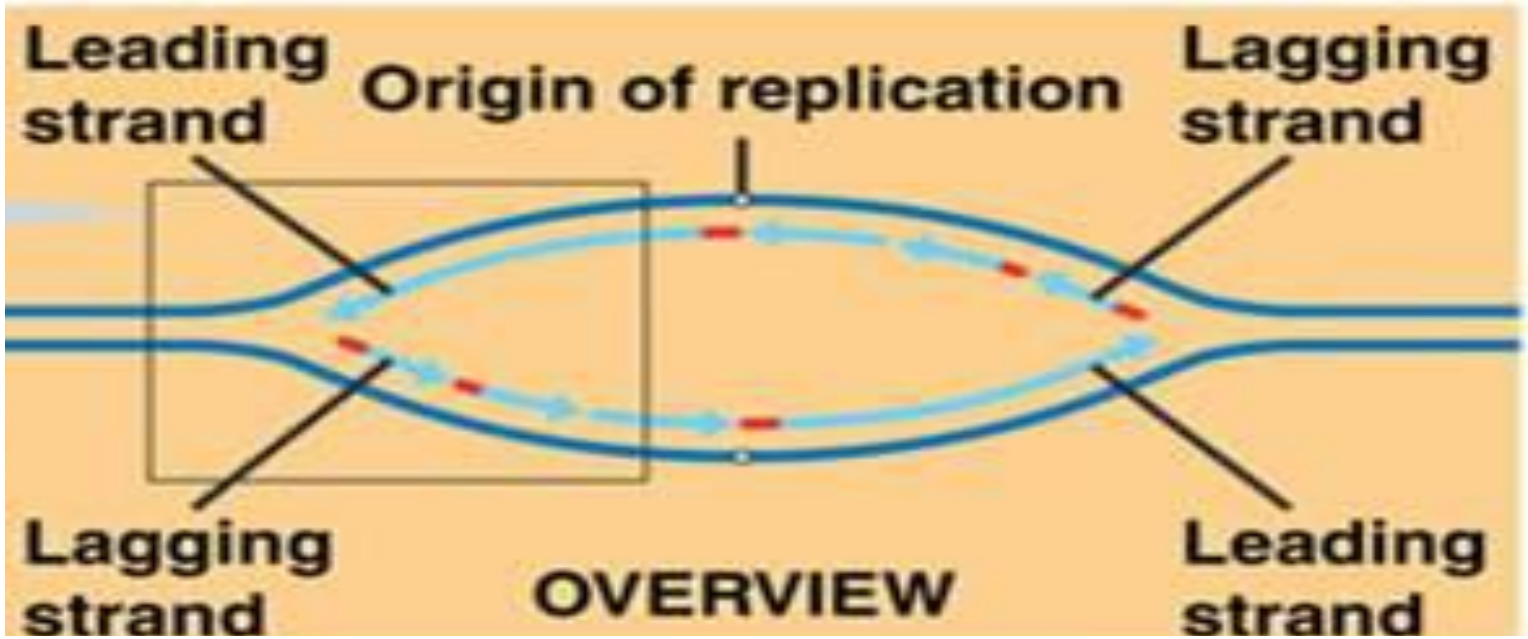
1- Initiation

- At the start of replication, replication fork forms when the parental strands of DNA unwind which allow to create a single stranded DNA template. Both DNA strands can act as templates to create two new identical DNA molecules



2- Elongation

- DNA synthesis or coping requires primer that is complementary in sequence to the DNA template strand.
- Two newly strands form, one strand is continuous (leading strand) and one is discontinuous short fragments (lagging strand or Okazaki fragment) that are joined together by DNA polymerase and ligase



3- Termination

- At the end of DNA replication, the RNA primers will be removed by the enzyme ribonuclease and double strand of DNA will be formed

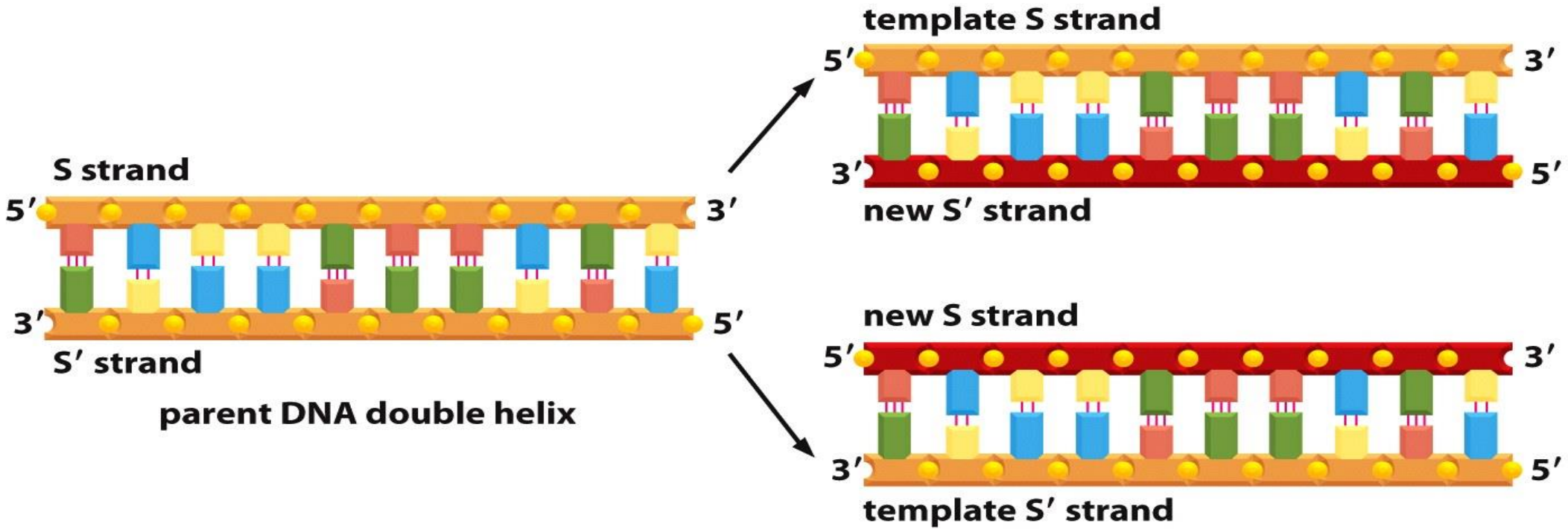


Figure 6-3 Essential Cell Biology 3/e (© Garland Science 2010)

DNA replication in prokaryotic and eukaryotic.

Prokaryotic replication	Eukaryotic replication
• semiconservative replication i.e. it produces two copies that each contain one of the original strands and one new strand	• semiconservative replication
• Bidirectional	• Bidirectional
• single origin replication (oriC)	• multiple origins of replication (ARS)
• primer synthesized by primase	• primer synthesized by subunits of DNA polymerase α
• processing enzyme: DNA polymerase III	• processing enzymes: DNA polymerases α and δ
• removal of primer: DNA polymerase I	• removal of primer: DNA polymerase β
• DNA free in cytoplasm as nucleoid	• DNA in nucleus arranges as chromatin structure
• circular DNA	• linear DNA

DNA Replication – why do you need to know?

1. Differences between eukaryotes and prokaryotes allow for targeted drugs
2. Understanding the role of changes involving DNA packaging in disease
3. Errors in replication can lead to genetic diseases
4. Increasing numbers of diagnostic tests based on DNA replication (PCR, sequencing etc)

Gene expression

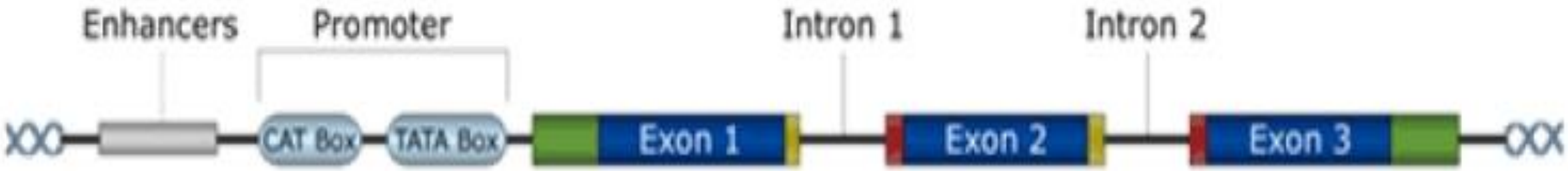
- Gene expression is the process by which the genetic code (the nucleotide sequence) of a gene (DNA) is used to direct protein synthesis.
- Genes that code for proteins are known as 'structural genes'.

Gene expression involves two main stages:

1- Transcription: Copying RNA from strand of DNA by enzyme RNA polymerases which occurs in the nucleus.

2- Translation: Using genetic information in the RNA to make a protein which happens in the cytoplasm.

Structural genes (DNA)

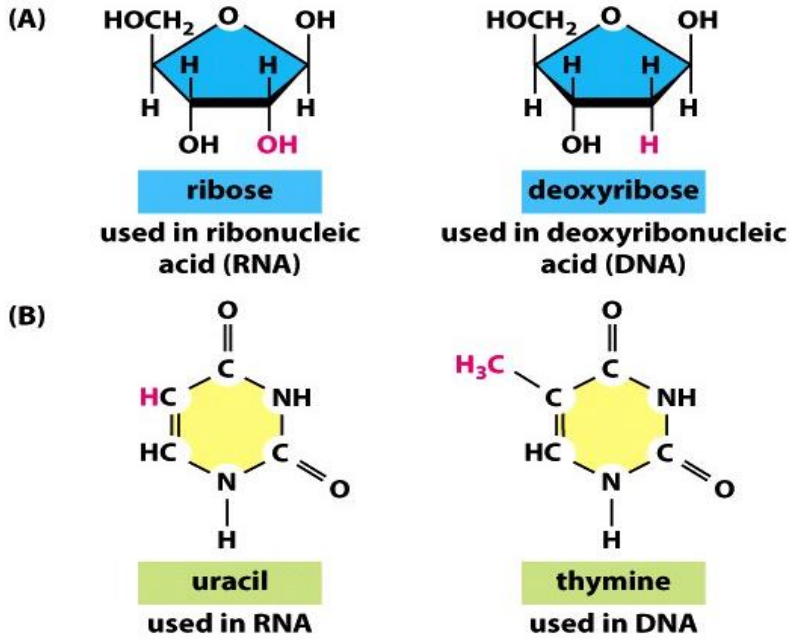


Transcription – making RNA

- Copying RNA from strand of DNA by enzyme RNA polymerases which occurs in the nucleus

Structure of RNA

- Single stranded molecule
- Uracil instead of thymine
- Ribose sugar instead of deoxyribose

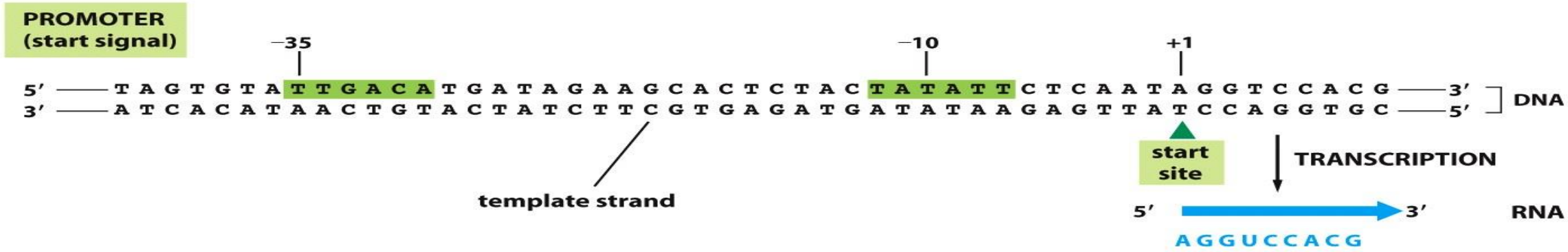


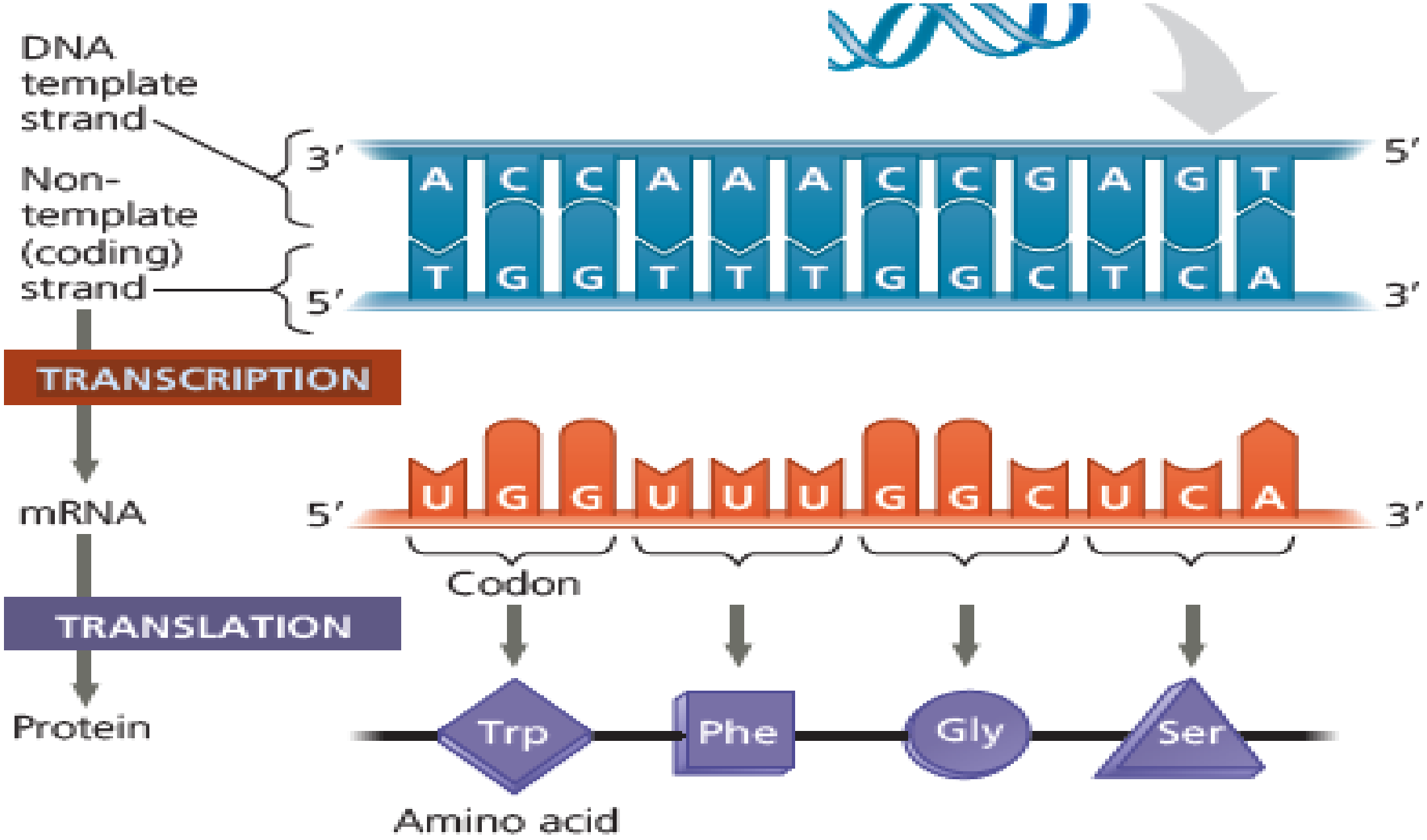
Steps of transcription

1- Initiation:

- Some proteins (initiation factors) will recognise the start of the gene and they will guide RNA polymerase where to start.
- The RNA polymerase will bind to the unwind DNA sequence at the **promoter** (a start signal)

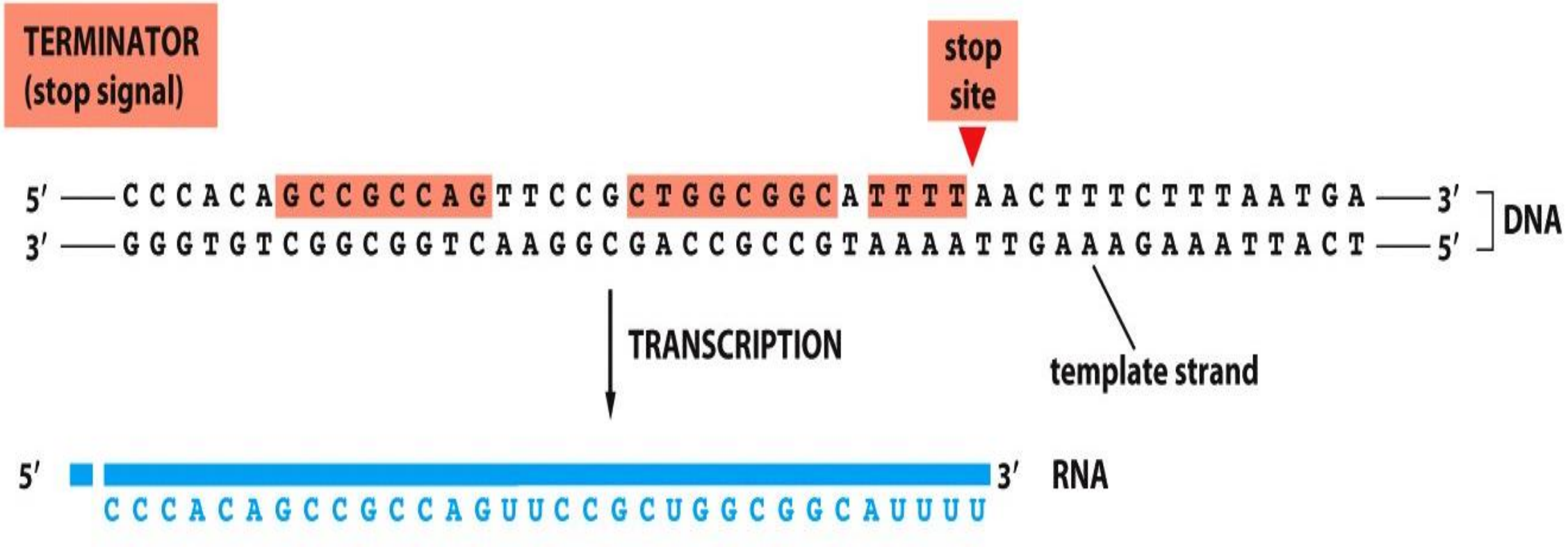
2- Elongation: Once the RNA polymerase binds, it runs along the DNA strand and starts transcribing from a start site by adding nucleotides




DNA template strand
Non-template (coding) strand
3′
5′
A C C A A A C C G A G T
T G G T T T G G C T C A
5′
3′
TRANSCRIPTION
mRNA
5′
U G G U U U G G C U C A
3′
Codon
TRANSLATION
Protein
Trp
Phe
Gly
Ser
Amino acid

3- Termination:

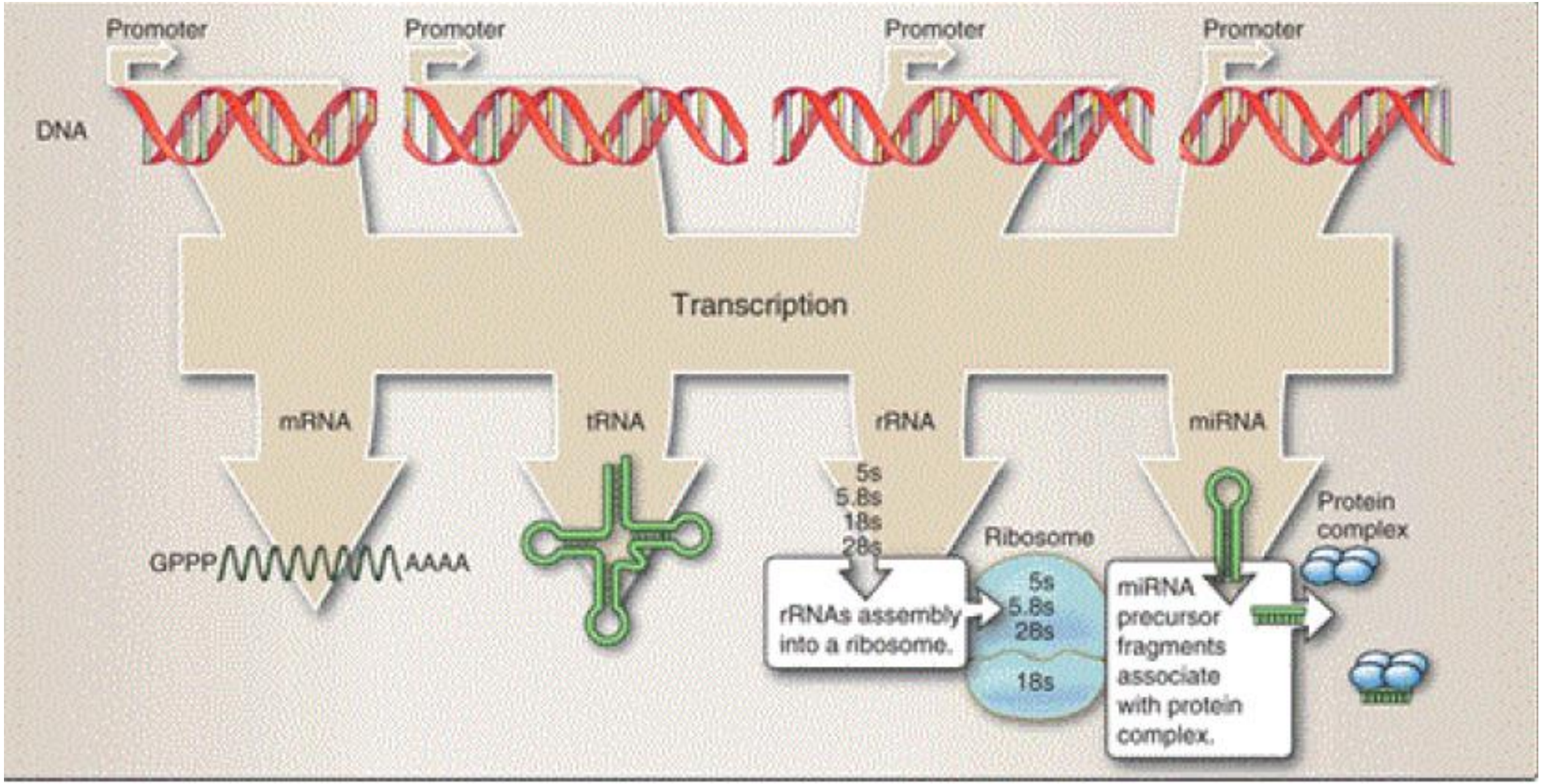
- Other proteins will recognise the end of the gene at terminator
- RNA polymerase will stop transcription at stop site and RNA will be formed.
- RNA will get out of the nucleus through nuclear pore to cytoplasm for protein synthesis



Types of RNA in a cell

1. **Messenger RNA (mRNA)**
   - It carries genetic information from DNA to encodes protein
   - Its about 5% of total RNA in the cell
2. **Transfer RNA (tRNA)**
   - Brings amino acids to the protein synthesising organelles called ribosomes
3. **Ribosomal RNA (rRNA)**
   - Makes up 80% of total RNA in the cell
   - Its part of the ribosomes
   - Plays an active role in recognizing codon and anticodon portions of mRNAs and tRNAs.

Four types of RNA in a cell



4. Non coding RNA (MicroRNA (miRNAs)

- miRNAs, are single-stranded non coding RNA, they are about 21 to 23 nucleotides in length.
- They function in regulating gene expression (coding protein)

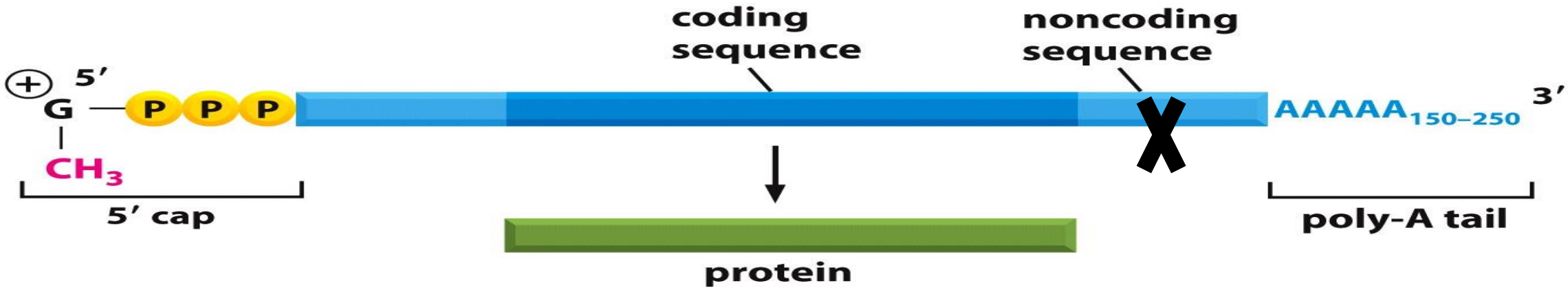
Three main types of RNA polymerase

1. **RNA polymerase I**
   - Synthesises large ribosomal RNAs
2. **RNA polymerase II**
   - mRNA and microRNA synthesis
3. **RNA polymerase III**
   - tRNA and 5S (small) rRNA synthesis

Processing of eukaryotic mRNA

- Transcript (mRNA) modified or processed so only the exons (coding sequences) remain because they contain the genetic information for protein synthesis.
- **After transcription, mRNA is processed by:**

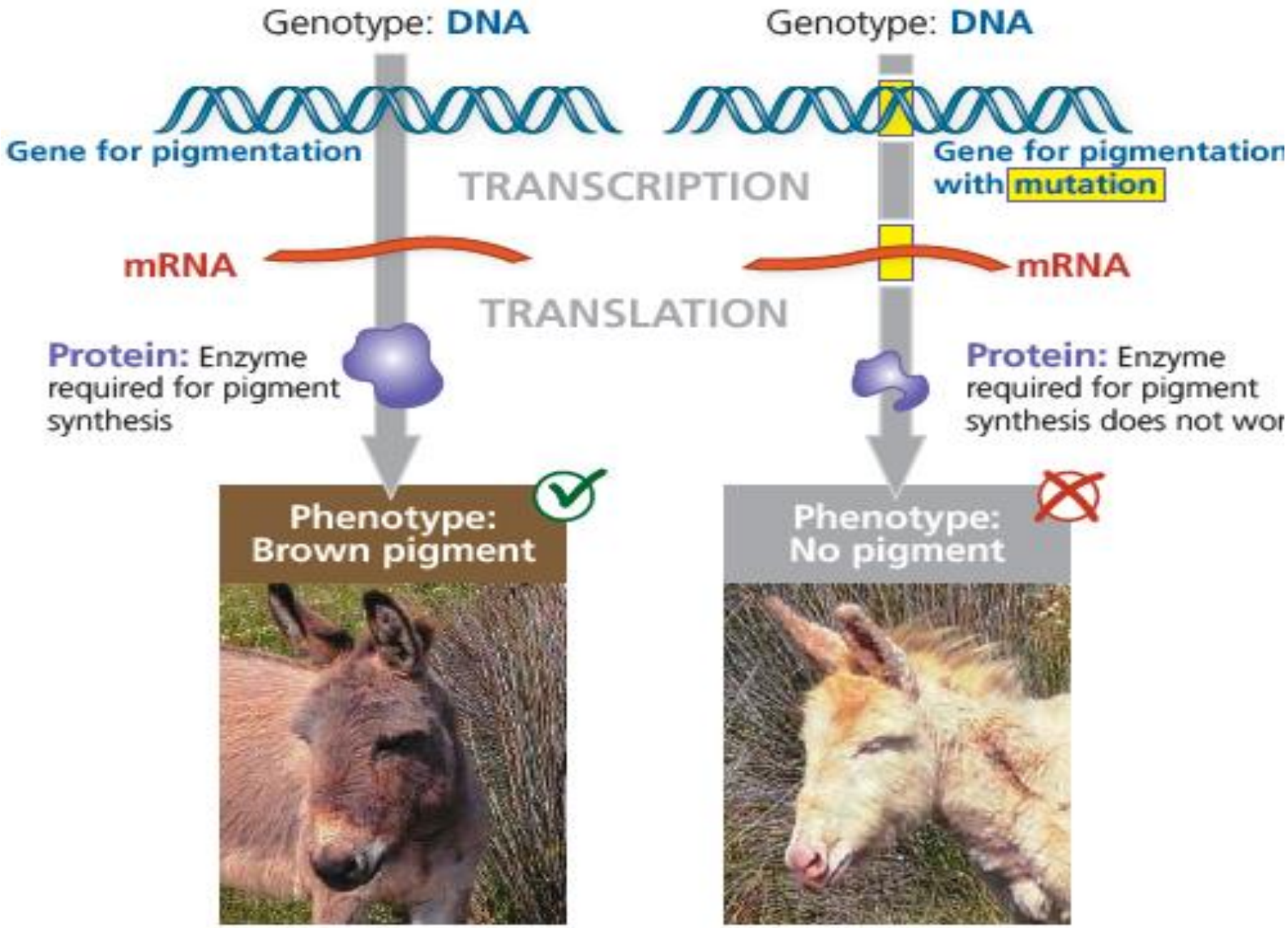
1. **Capping (addition of modified G to 5’ end)**
2. **Splicing (removal of introns (noncoding sequence)**
3. **Polyadenylation (addition of A to 3’ end) called poly-A-tail**



Causes of DNA mutation

DNA can be damaged by:

- Chemical mutagens: Industrial chemicals such as vinyl chloride and hydrogen peroxide, and environmental chemicals such as polycyclic hydrocarbons found in smoke, soot and tar
- Radiation: X-rays and radioactive radiation
- Free radicals: UV-A light creates free radicals


Genotype: DNA
Gene for pigmentation
TRANSCRIPTION
mRNA
TRANSLATION
Protein: Enzyme required for pigment synthesis
Phenotype: Brown pigment
Genotype: DNA
Gene for pigmentation with mutation
mRNA
Protein: Enzyme required for pigment synthesis does not wor
Phenotype: No pigment