EUKARYOTIC GENE EXPRESSION

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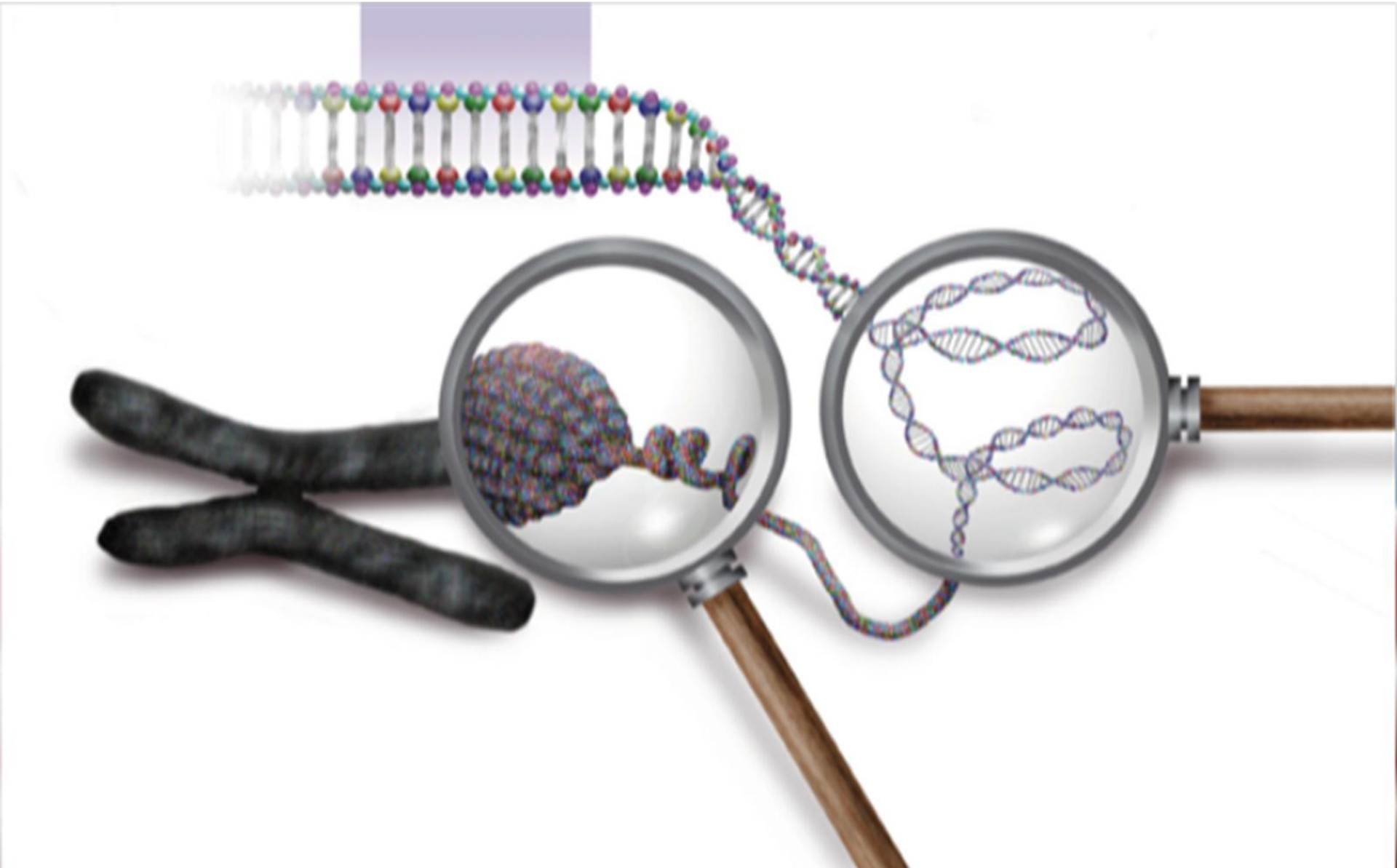


Target Audience

- Medical students;
- Medical technologist;
- Beginners and
- For every laboratory worker and everyone passion for learning.

CONTENTS

- The Gene Structure
- Gene Expression
- Transcription
- Genetic Code and Protein Synthesis
- Regulation of Gene Expression

GENE EXPRESSION

Gene expression which a gene's information is converted into the structures and functions of a cell.

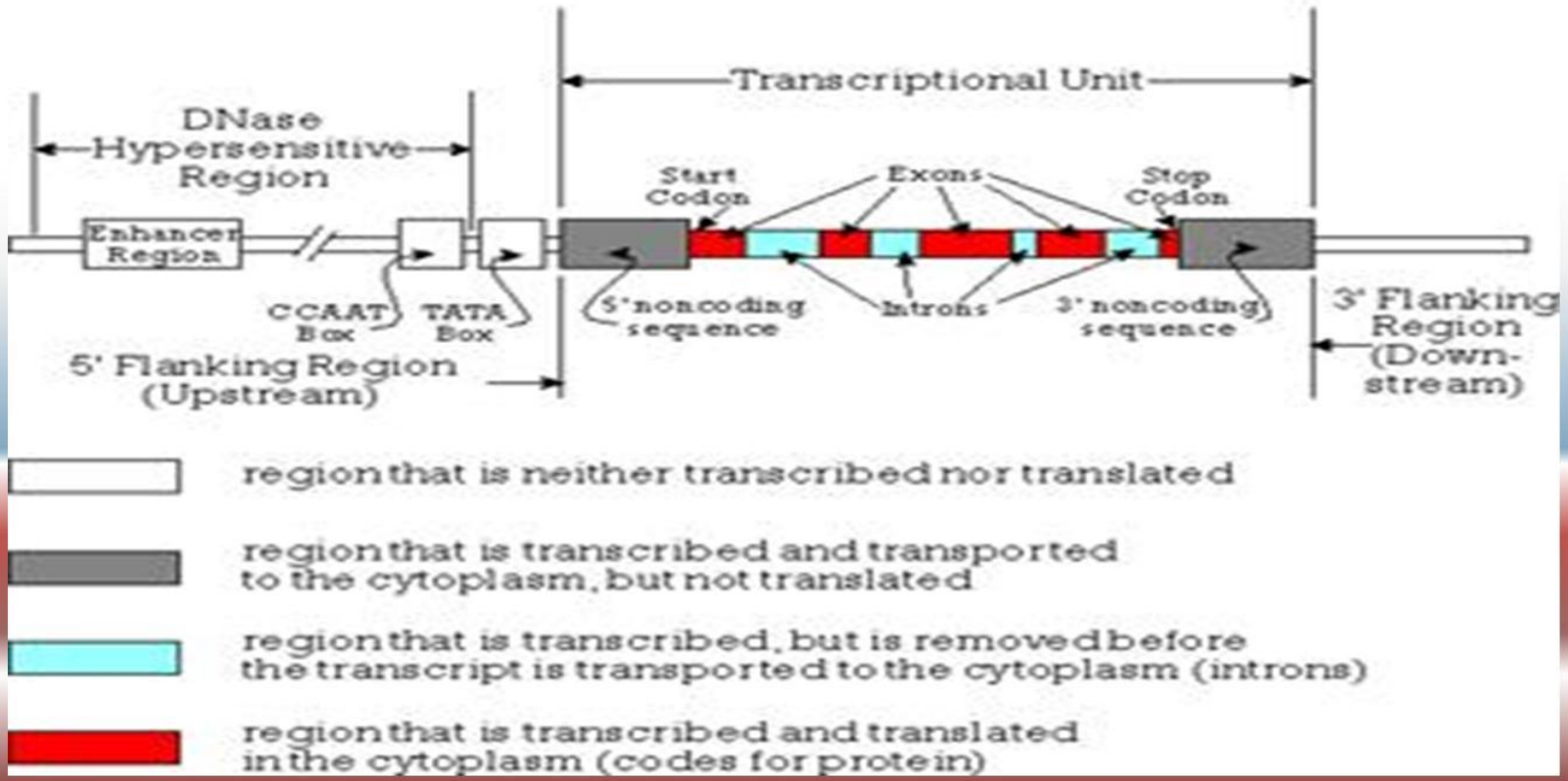
Gene expression; the process of producing a biologically functional molecule of either protein or RNA (gene product) is made.

Gene expression is assumed to be controlled at various points in the sequence leading to protein synthesis.

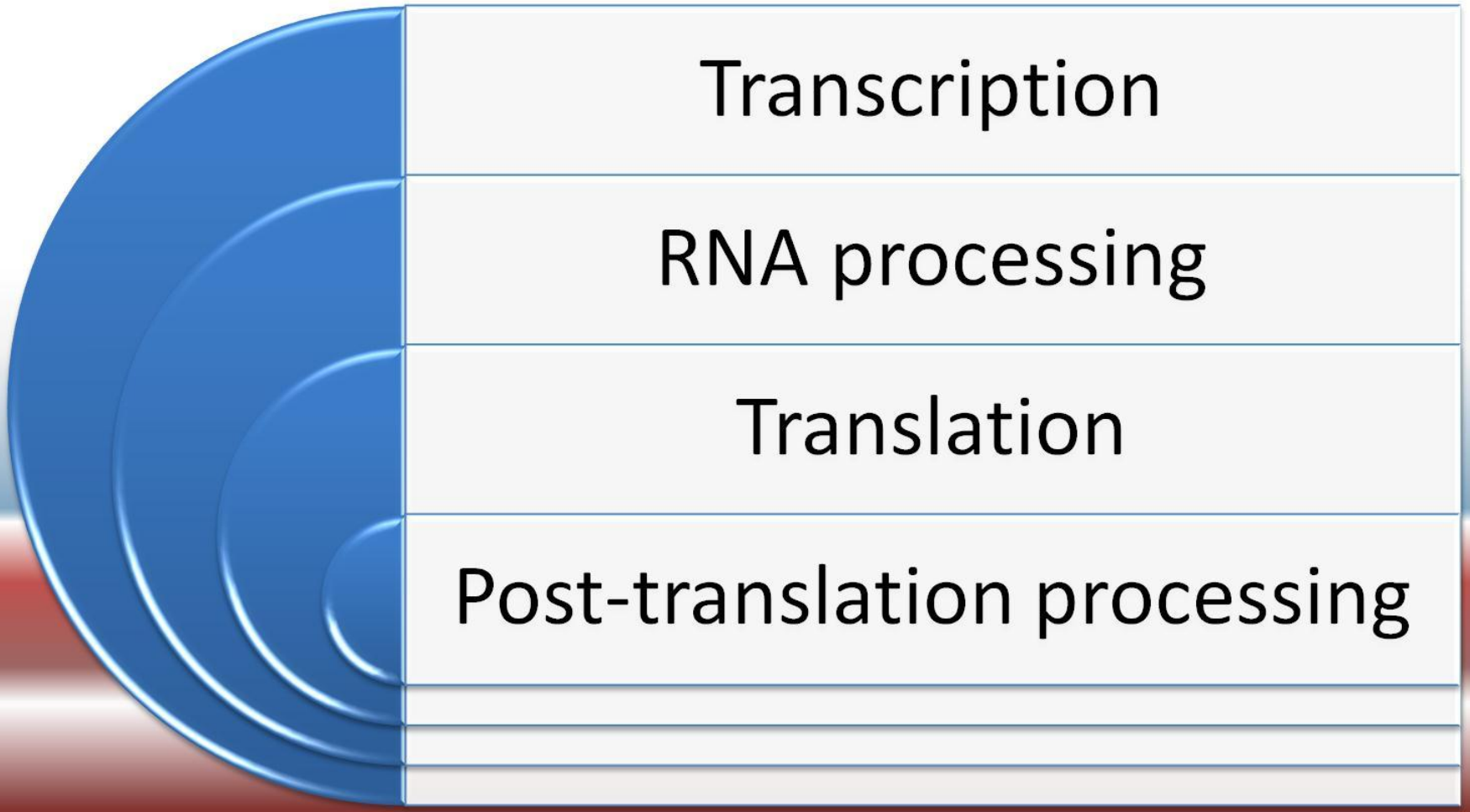
GENE STRUCTURE

- Eukaryotic gene structure:
  - The average gene 7-10 exons spread over 10-16kb of DNA.
  - The gene must have (Exon; start signals; stop signals; regulatory control elements).
  - Gene structure
    - Exons (The coding sequences)
    - Introns (The non coding sequences).

GENE STRUCTURE



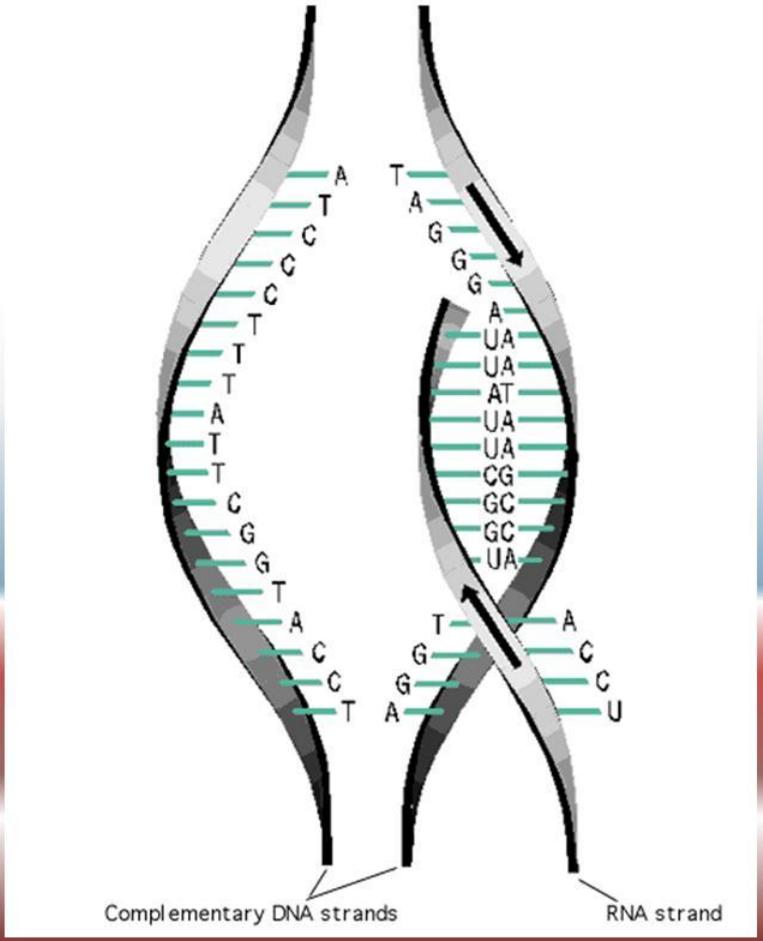
GENE EXPRESSION



TRANSCRIPTION

Transcription

- Synthesis of an RNA that is complementary to one of the strands of DNA.



Requirement for Transcription

- Promoter
- Enhancer
- Transcription Enzymes
  - RNA polymerase
- Transcription Factors
- Mediators
- Activators

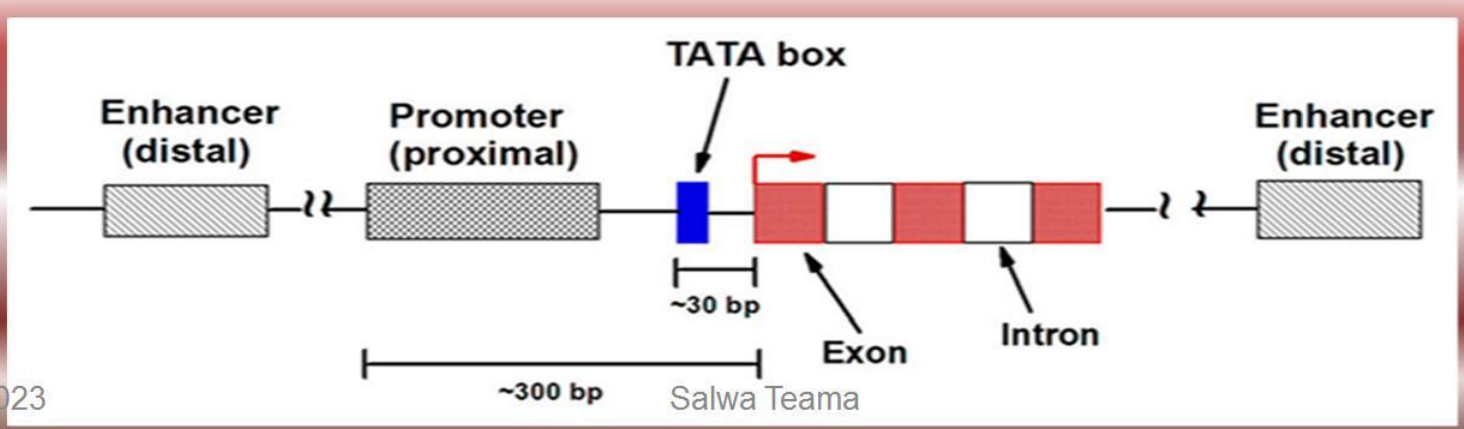
Eukaryotic Promoter

Eukaryotic Promoter lies upstream of the gene. There are several different types of promoter found in human genome, with different structure and different regulatory properties class/I/II/III.

Conserved eukaryotic promoter elements	Consensus sequence
CAAT box	GGCCAATCT
TATA box	TATAA
GC box	GGGCGG
CAP site	TAC

Enhancers

Enhancers are stretches of bases within DNA, about 50 to 150 base pairs in length; the activities of many promoters are greatly increased by **enhancers** which can exert their stimulatory actions along several thousands base pairs.



Transcription Enzymes

RNA polymerase: The enzyme that controls transcription and is characterized by:

- Search DNA for initiation site,
- It unwinds a short stretch of double helical DNA to produce a single-stranded DNA template,
- It selects the correct ribonucleotide and catalyzes the formation of a phosphodiester bond,
- It detects termination signals where transcript ends.

Eukaryotic RNA polymerases have different roles in transcription

Polymerase I	Nucleolus	Makes a large precursor to the major rRNA (5.8S,18S and 28S rRNA in vertebrates
Polymerase II	Nucleoplasm	Synthesizes hnRNAs, which are precursors to mRNAs. It also make most small nuclear RNAs (snRNAs
Polymerase III	Nucleoplasm	Makes the precursor to 5SrRNA, the tRNAs and several other small cellular and viral RNAs.

Transcription Factors

- Transcription factors are proteins that bind to DNA near the start of transcription of a gene.

- Transcription factors either inhibit or assist RNA polymerase in initiation and maintenance of transcription.

Mediators, another collection of proteins and can be considered as a general transcription factor, because it is a part of class II preintiation complexes. Mediator is not required for initiation per se, but it is required for activated transcription.

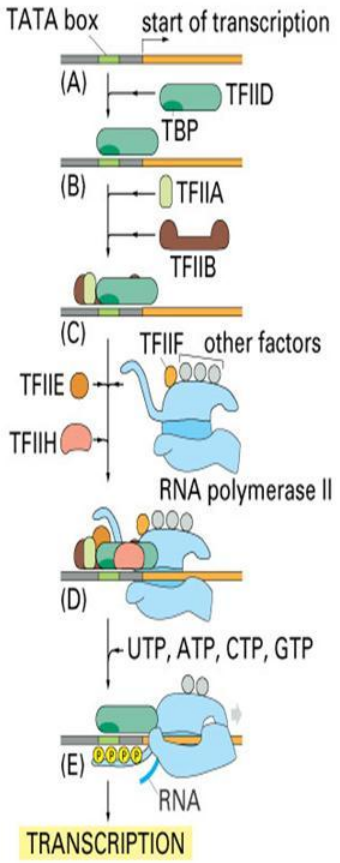
Activators (gene specific transcription factors) can provide more activation in transcription.

Activators can bind to enhancers and also permits cells to control expression of their genes.

Eukaryotic activators recruit RNA polymerase to promoters and stimulate binding of general transcription factor and RNA to the promoter.

TRANSCRIPTION

- The assembly of the preinitiation complex on each kind of eukaryotic promoter (class II promoters recognized by RNA polymerase II) begins with the binding of an assembly factor to the promoter.



Preparatory Steps for Transcription

Preinitiation complex

- Core Promoter Sequence
- Transcription Factors
- RNA Polymerase
- Activators and Repressors.

TRANSCRIPTION

- The polymerase binding causes the unwinding of the DNA double helix which expose at least 12 bases on the template.
- This is followed by initiation of RNA synthesis at this starting point.
- The RNA polymerase starts building the RNA chain; it assembles ribonucleotides triphosphates: ATP; GTP; CTP and UTP into a strand of RNA.
- After the first nucleotide is in place, the polymerase joins a second nucleotide to the first, forming the initial phosphodiester bond in the RNA chain.

TRANSCRIPTION

- **RNA polymerase** directs the sequential binding of ribonucleotides to the growing RNA chain in the 5' - 3' direction.
- Each ribonucleotide is inserted into the growing RNA strand following the rules of base pairing. This process is repeated till the desired RNA length is synthesized.
- Terminators at the end of genes; signal termination. the association between RNA product and DNA template loosen with RNA polymerase, the RNA dissociate from RNA polymerase and DNA and stop transcription.

TRANSCRIPTION

Primary transcript (immature RNA or pre mRNA).

- The primary product of RNA transcription; the hnRNAs contain both intronic and exonic sequences.
- These hnRNAs are processed in the nucleus to give mature mRNAs that are transported to the cytoplasm where to participate in protein synthesis.

RNA Processing (Pre-mRNA → mRNA)

Capping

The cap structure is added to the 5' of the newly transcribed mRNA precursor in the nucleus prior to processing and subsequent transport of the mRNA molecule to the cytoplasm.

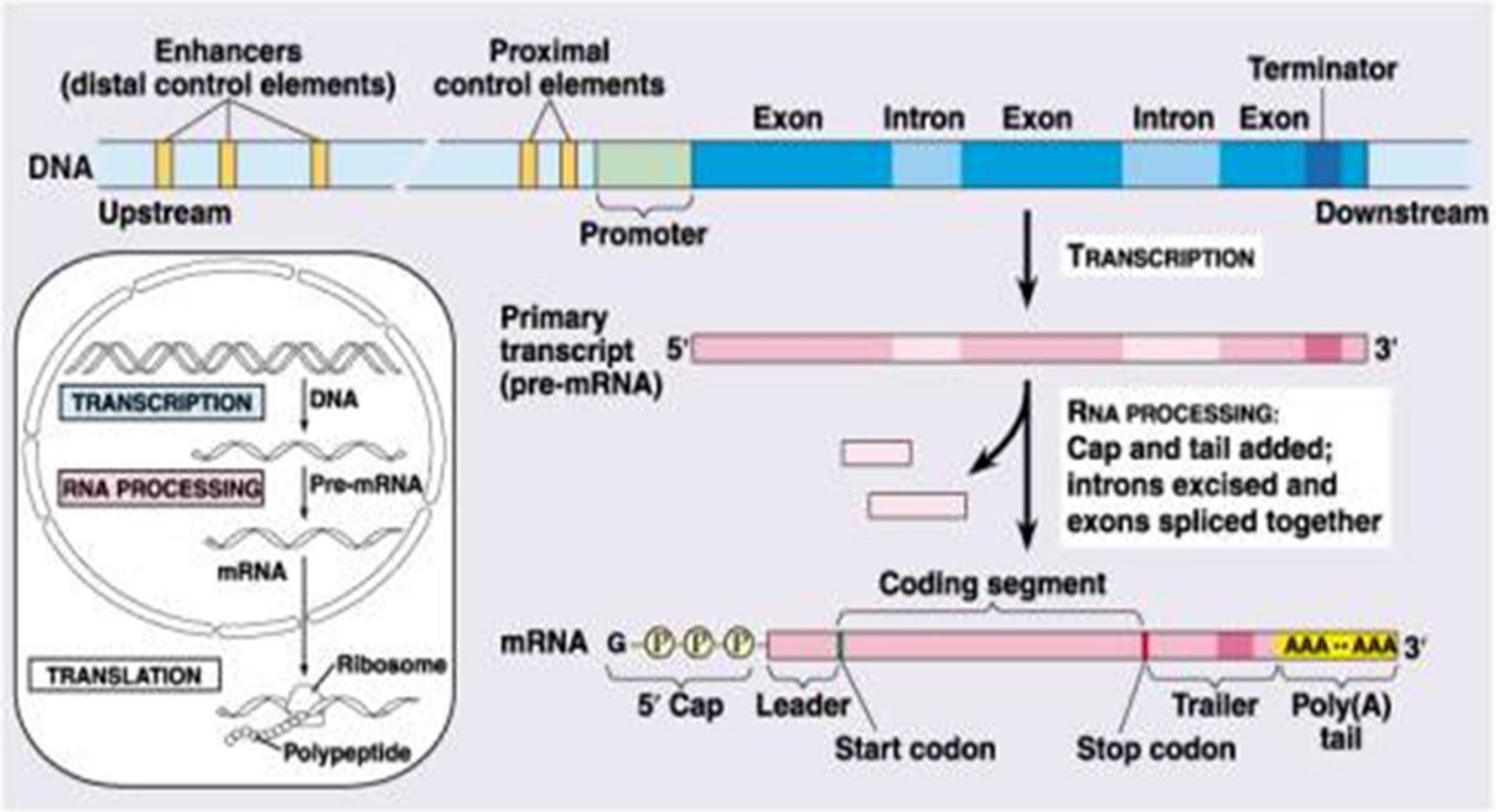
Splicing

Joining of exons; it takes place on a special structure called spliceosomes.

Addition of poly A tail

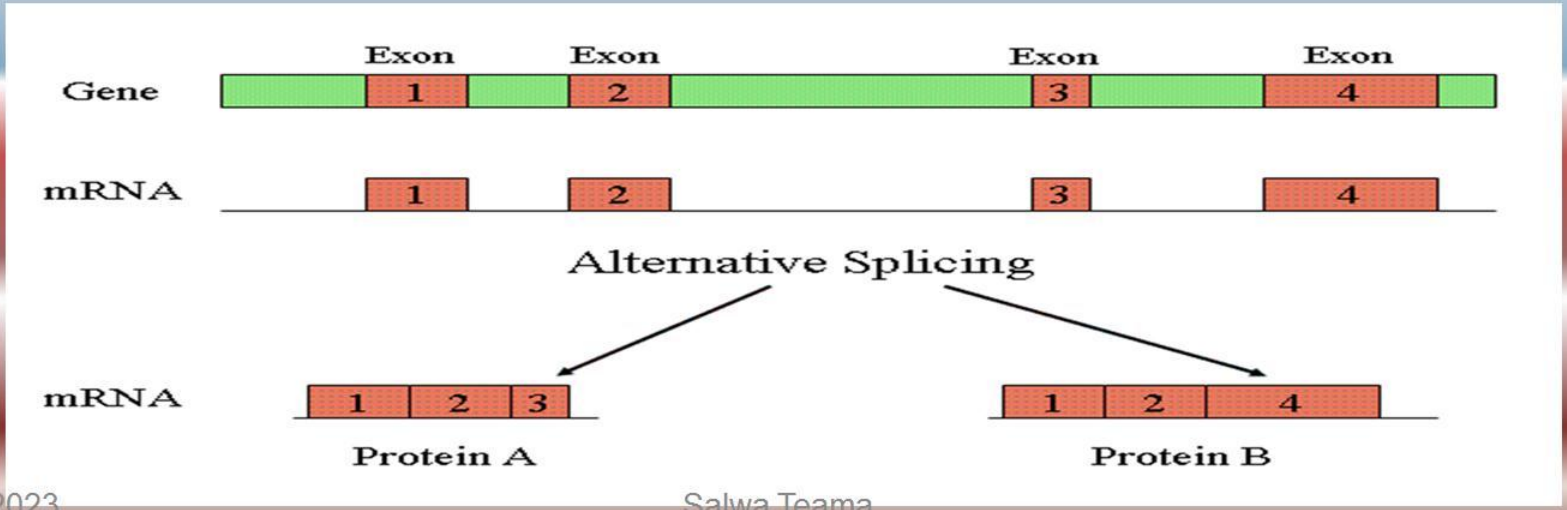
Synthesis of the poly (A) tail involves cleavage of its 3' end and then the addition of about 40- 200 adenine residues to form a poly (A) tail.

TRANSCRIPTION



ALTERNATIVE SPLICING

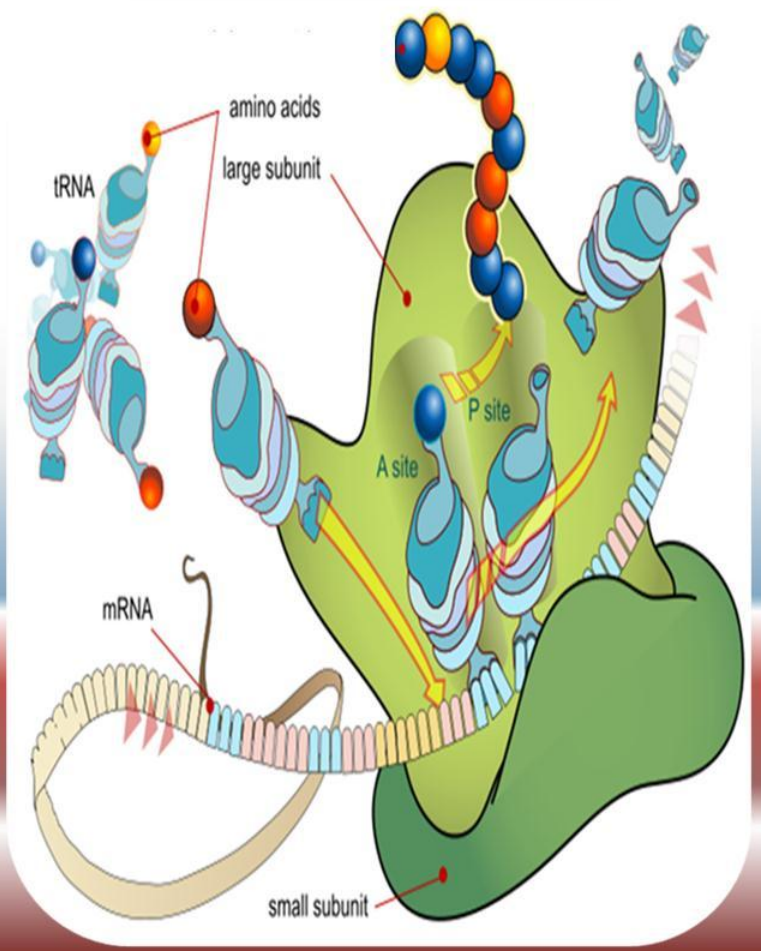
Alternative splicing: is a very common phenomenon in higher eukaryotes. It is a way to get more than one protein product out of the same gene and a way to control gene expression in cells.



TRANSLATION

Translation

- Ribosomes read a messenger RNA and make protein according to its instruction.



Requirement for Translation

- Ribosomes
- tRNA
- mRNA
- Amino acids
- Initiation factors
- Elongation factors
- Termination factors
- Aminoacyl tRNA synthetase enzymes
- Energy source

Preparatory Steps for Protein Synthesis

The protein synthesis occur in 3 phases:

- Intiation
- Elongation
- Termination

First, aminoacyl tRNA synthetase joins amino acid to their specific tRNA.

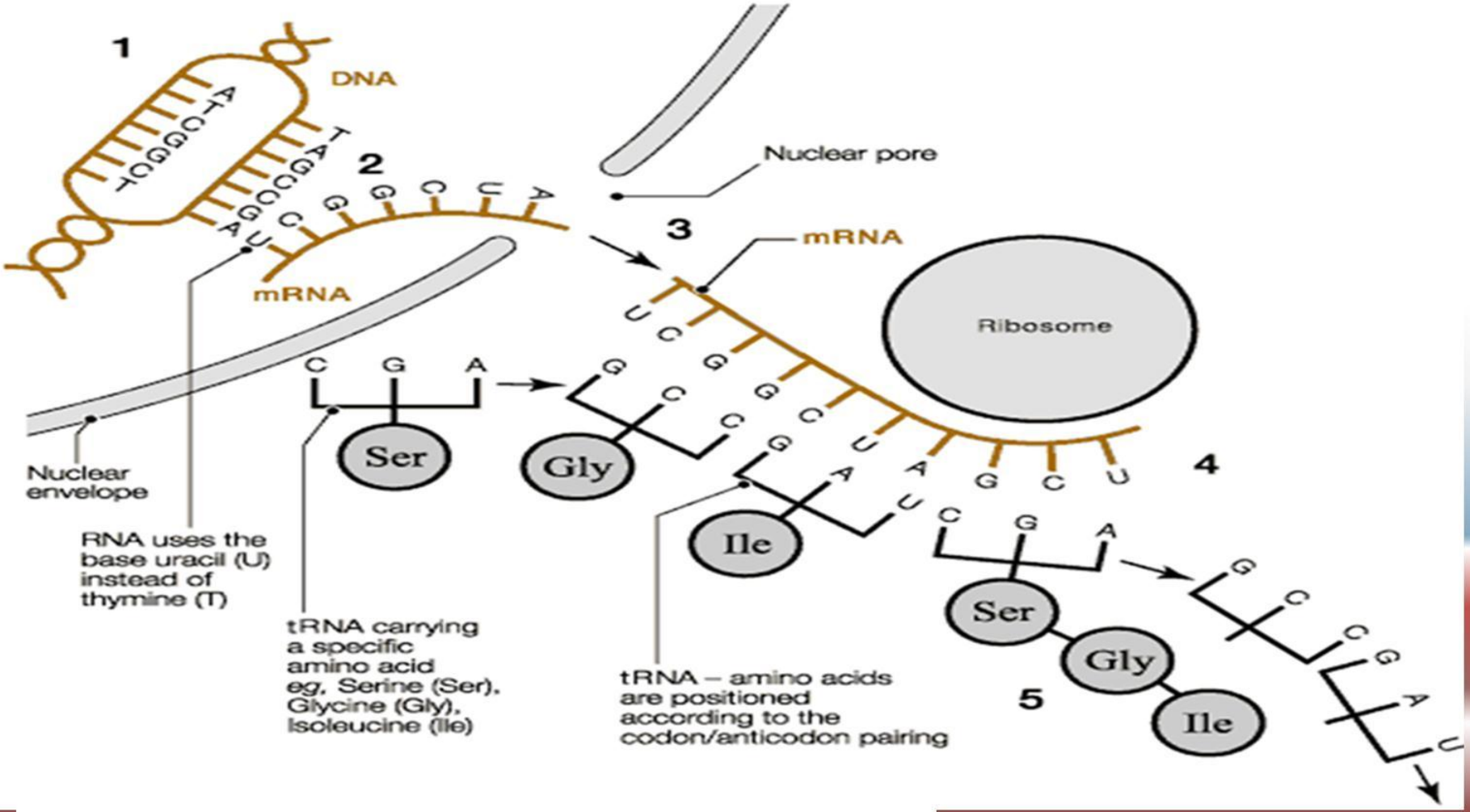
Second, ribosomes must dissociate into subunits at the end of each round of translation.

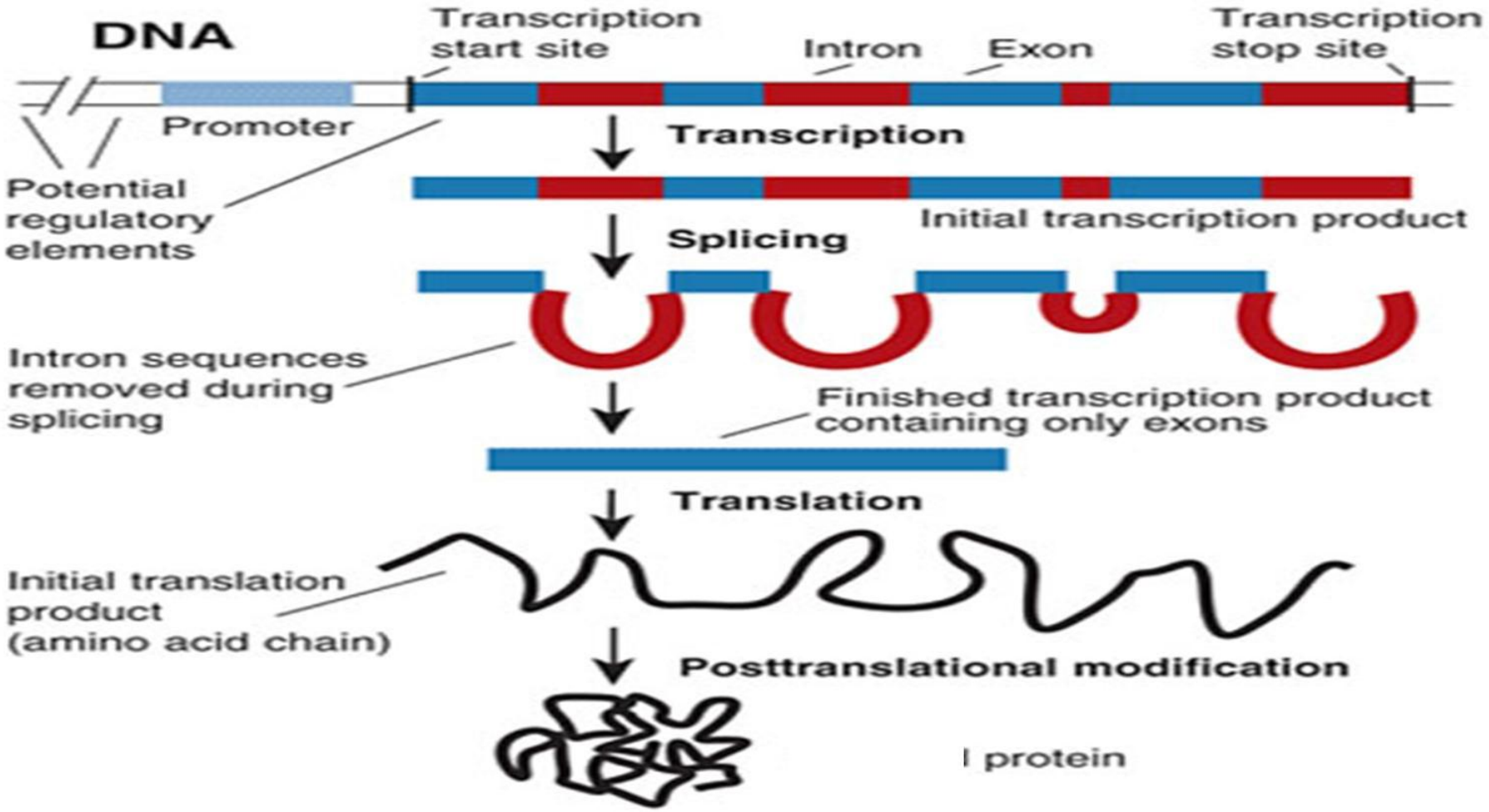
TRANSLATION

- Accurate and efficient initiation occurs; the ribosomes binds to the mRNA, and the first amino acid attached to its tRNA.
- The initiation phase of protein synthesis requires over 10 eukaryotic Initiation Factors (eIFs): Factors are needed to recognize the cap at the 5' of mRNA and binding to the 40s ribosomal subunit.
- Binding the initiator Met-tRNAiMet (methionyl- tRNA) to the 40S small subunit of the ribosome.
- Scanning to find the start codon by binding to the 5'cap of the mRNA and scanning downstream until they find the first AUG (initiation codon).
- The start codon must be located and positioned correctly in the P site of the ribosome, and the initiator tRNA must be positioned correctly in the same site.
- Once the mRNA and initiator tRNA are correctly bound, the 60S large subunit binds to form 80s initiation complex with a release of the eIF factors.

TRANSLATION

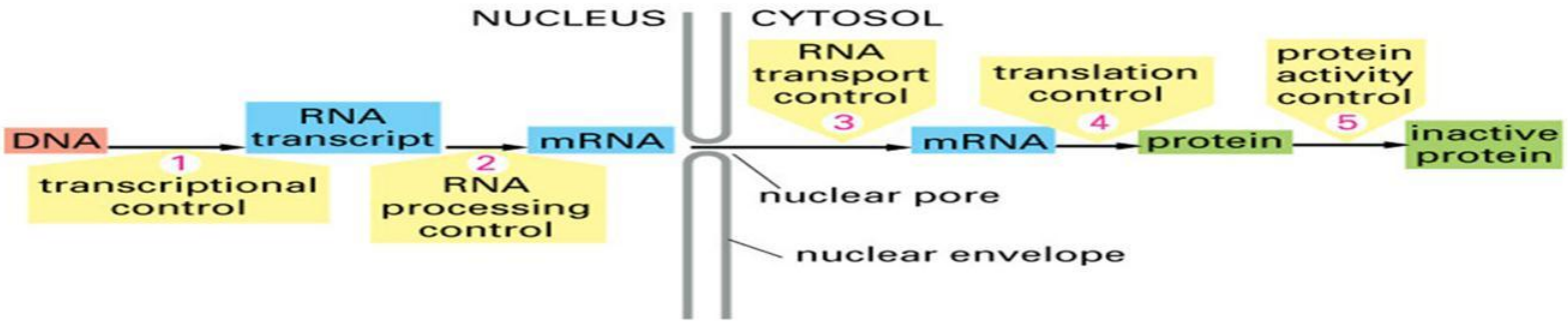
- Chain elongation, the ribosomes adds one amino acid at a time to the growing polypeptide chain.
- **Transfer of proper aminoacyl-tRNA from cytoplasm to A-site of ribosome;**
- **Peptide bond formation**; Peptidyl transferase forms a peptide bond between the amino acid in the P site, and the newly arrived aminoacyl tRNA in the A site. This lengthens the peptide by one amino acids.
- Translation termination requires specific protein factors identified as releasing factors (eRFs) in eukaryotes; termination signal (nonsense codon of mRNA) which cause the release of the synthesized protein.


1
DNA
2
mRNA
Nuclear pore
3
mRNA
Ribosome
Nuclear envelope
RNA uses the base uracil (U) instead of thymine (T)
tRNA carrying a specific amino acid eg, Serine (Ser), Glycine (Gly), Isoleucine (Ile)
Ser
Gly
Ile
tRNA – amino acids are positioned according to the codon/anticodon pairing
4
5
Ser
Gly
Ile


DNA
Transcription start site
Intron
Exon
Transcription stop site
Promoter
Transcription
Potential regulatory elements
Initial transcription product
Splicing
Intron sequences removed during splicing
Finished transcription product containing only exons
Translation
Initial translation product (amino acid chain)
Posttranslational modification
l protein

CONTROL OF GENE EXPRESSION

- Transcriptional
- Posttranscriptional
- Translational
- Posttranslational



Eukaryotic Gene Expression

- Essentially all humans' genes contain introns. A notable exception is the histone genes which are intronless.
- Eukaryote genes are not grouped in operons. Each eukaryote gene is transcribed separately, with separate transcriptional controls on each gene.
- Eukaryotic mRNA is modified through RNA splicing.
- Eukaryotic mRNA is generally monogenic (monocistronic); code for only one polypeptide.

Eukaryotic Gene Expression

- Eukaryotic mRNA contain no Shine-Dalgarno sequence to show the ribosomes where to start translating. Instead, most eukaryotic mRNA have caps at their 5` end which directs initiation factors to bind and begin searching for an initiation codon.
- Eukaryotes have a separate RNA polymerase for each type of RNA.
- In eukaryotes, polysomes are found in the cytoplasm.
- Eukaryotic protein synthesis initiation begins with methionine not N formyl- methionine.

Prokaryotic vs. Eukaryotic

- Bacterial genetics are different.
- Prokaryote genes are grouped in operons.
- Prokaryotes have one type of RNA polymerase for all types of RNA,
- mRNA is not modified
- The existence of introns in prokaryotes is extremely rare.

Prokaryotic vs. Eukaryotic

- To initiate transcription in bacteria, sigma factors bind to RNA polymerases. RNA polymerases/ sigma factors complex can then bind to promoter about 40 deoxyribonucleotide bases prior to the coding region of the gene.
- In prokaryotes, the newly synthesized mRNA is polycistronic (polygenic) (code for more than one polypeptide chain).
- In prokaryotes, transcription of a gene and translation of the resulting mRNA occur simultaneously. So many polysomes are found associated with an active gene.


THANK YOU