

# **Assignment**

## **Cellular perspective**

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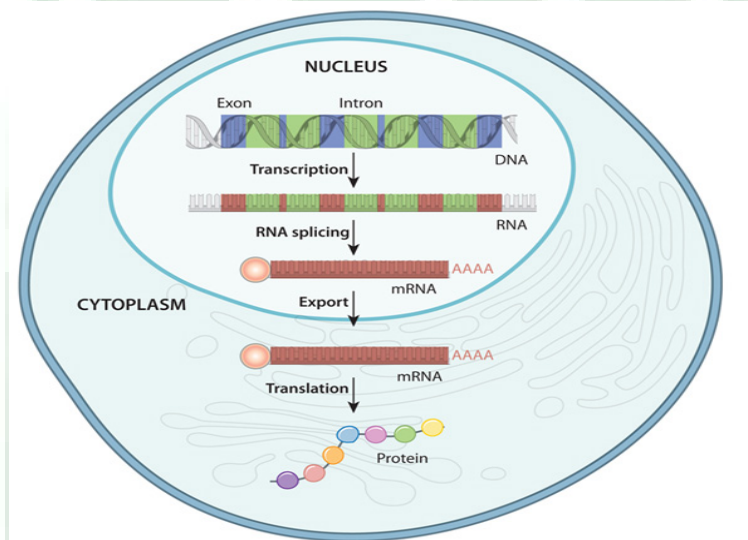
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# Gene expression:

- Gene expression is first formulated by **Francis Crick** in **1958**.
- It is also formulated as **Central Dogma**.

## ❖ Definition:

It is a process in which formation of a gene is used in the synthesis of functional gene product.



## ❖ Occurrence:

This process is used by all known life like **eukaryotes**, **prokaryotes** and **viruses** - to generate molecular machinery of life.

## ❖ Structural genes:

“Genes that code for amino acids sequence are called structural genes”.

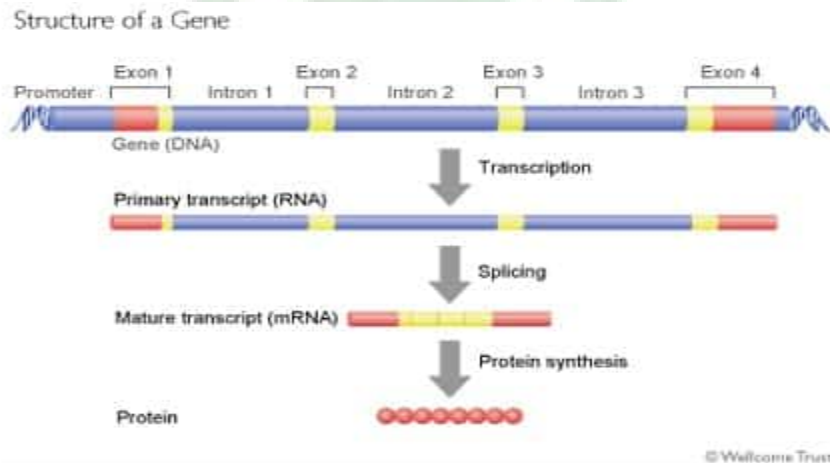
Structural genes have different components:

- Exons:

Exons code for amino acids and collectively determine amino acid sequence of protein product.

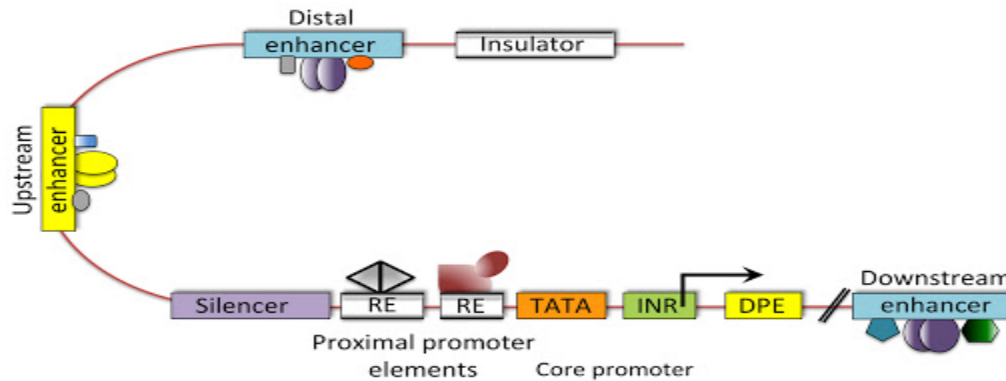
- **Introns:**

Introns are nucleotide sequences in DNA and RNA that do not directly code for proteins, and are removed during the precursor messenger RNA (pre-mRNA) stage of maturation of mRNA by RNA splicing.



### ❖ **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.



### ❖ Mechanism:

The process of gene expression includes the following steps:

- **Transcription** - Transcription is the process by which a segment of DNA is used to generate an RNA template. The DNA segment is “read” by an enzyme called RNA polymerase, which produces a strand of RNA that is complimentary to the DNA. In this complementary RNA strand, all thymine bases are replaced by uracil.
- **Processing** - This primary RNA transcript is then modified to convert it into mature messenger RNA (mRNA) that can be used in translation. The mRNA undergoes splicing to remove the non-coding parts of the transcript (introns) so that only the coding sections (exons) remain.
- **Non-coding RNA maturation** - Non-coding regions of RNA (ncRNA) are transcribed as precursors which are then processed further. For example, these regions may be transcribed as pre-ribosomal RNA (pre-rRNA) which then undergoes cleavage to become ribosomal RNA (rRNA).
- **RNA export** - The majority of mature RNA is then transported from the nucleus to the cytoplasm. Although some RNAs function in the nucleus, most are carried through pores in the nucleus into the cytosol, including all RNAs involved in protein synthesis.

- **Translation** - The final mRNA carries the information needed to code for proteins. Every three base pairs on the mRNA corresponds to a binding site for a transfer RNA (tRNA) which carries an amino acid. The amino acids are then linked together in a chain by a ribosome to create a rudimentary protein chain.
- **Protein folding** - The long chain of amino acids folds to form a three-dimensional structures using enzymes called chaperones. This three-dimensional structure is the final, functional form of the protein.

### ❖ Gene regulation:

“Gene regulation refers to mechanism that acts to induce or repress the expression of gene”.

These include structural and chemical changes to the genetic material, binding of proteins to specific DNA elements to regulate transcription, or mechanisms that modulate translation of mRNA.

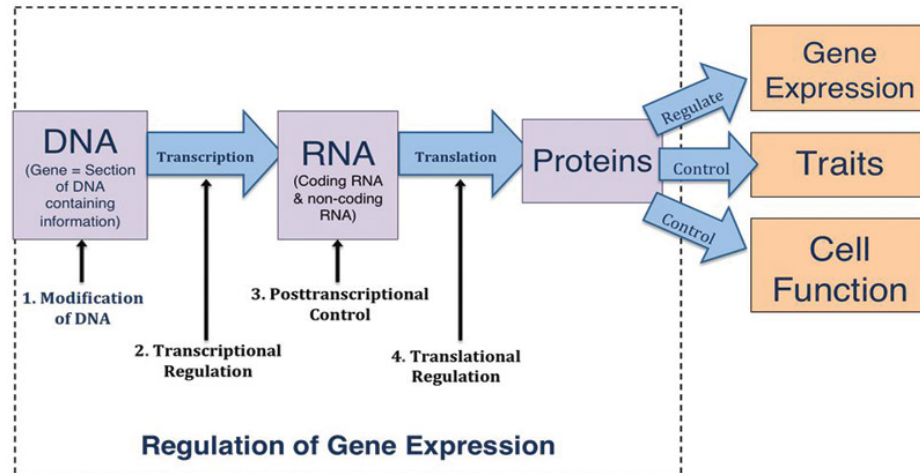
#### ➤ Mechanism of gene regulation:

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.

#### ➤ Control of gene expression:

- Transcription
- Post transcription
- Translation
- Post translation



### ➤ Factors:

It depends upon various factors:

- Chromosomal activation or deactivation
- Control of initiation of transcription
- RNA splicing
- Control of mRNA transport
- Control of mRNA degradation
- Control of initiation of translation(Eukaryotes)
- Post translational modification

### ➤ Gene regulation in eukaryotes and prokaryotes:

- Eukaryotic mRNA is modified through RNA splicing while prokaryotic mRNA is not modified.
- Eukaryotic mRNA is monogenic (code for one polypeptide) while Prokaryotic mRNA is polygenic (code for more than one polypeptide)
- Prokaryotic genes are grouped into operons while eukaryotic genes are not grouped.
- Prokaryotes have single RNA polymerase while eukaryotes have 3 types of

# Transcription:

## ❖ Definition:

Transcription is the process in which a gene's DNA sequence is copied (transcribed) to make an RNA molecule with the help of enzyme **RNA polymerase**.

## ❖ Explanation:

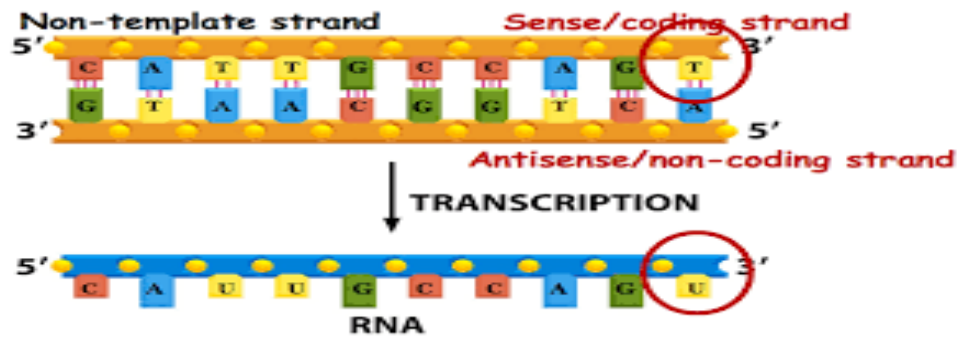
The genetic information in DNA is not translated directly into protein. Transcription is first step of gene expression. During this process, the DNA sequence of a gene is copied into RNA. Before transcription can take place, the DNA double helix must unwind near the gene that is getting transcribed. The region of opened-up DNA is called a **transcription bubble**.

## ❖ Sites of transcription:

- **Prokaryotes**\_\_cytoplasm (all RNAs )
- **Eukaryotes**\_\_ nucleus and mitochondria
  - ◆ Nucleolus \_\_ r RNA
  - ◆ Nucleoplasm \_\_tRNA and m RNA

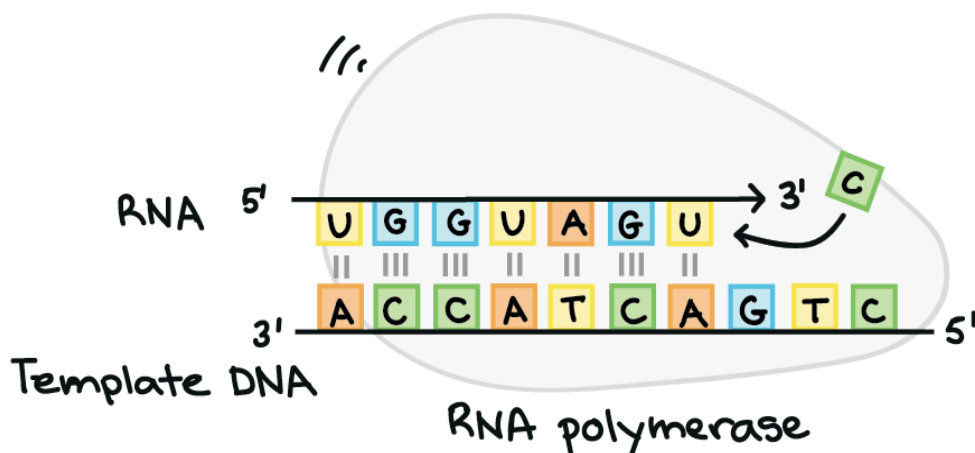
## ❖ Transcriptional traits:

- Transcription is highly selective. This is due to signals embedded in nucleotide sequence of DNA. These signals regulate the initiation and termination of transcription.
- Many of RNA transcripts are synthesized as precursors known as **primary transcripts**.
- Many enzymes are involved in transcription. Which perform following functions
  - They unwind a region of a DNA molecule.
  - They initiate and end mRNA synthesis.
  - They also modify mRNA after completion of transcription.
- However **RNA polymerase** is main enzyme involved in transcription. RNA polymerase uses one of DNA strand as a template on which complimentary ribonucleotides are incorporate to synthesize RNA.
- The strand of DNA which is transcribed to RNA is called as **template strand** (antisense/noncoding). Opposite strand is called as **coding strand** (sense / non template)



### ❖ RNA polymerase:

RNA polymerases are enzymes that transcribe DNA into RNA. Using a DNA template, RNA polymerase builds a new RNA molecule through base pairing. RNA polymerase builds a strand in 5' to 3' direction and add nucleotides in 3' end of chain.



### ➤ Types of RNA polymerase:

- Prokaryotes have only one type of RNA polymerase
- Eukaryotes have 3 types of RNA polymerase
  - **RNA polymerase** : It is located in nucleolus and transcribes r RNA.
  - **RNA polymerase** : It is localized to nucleus. It transcribes m RNA and most small nuclear RNAs (snRNAs).
  - **RNA polymerase** : It is also localized to nucleus. It transcribes t RNA and other small RNAs (5S r RNAs)

### ➤ Function of RNA polymerase:

RNA polymerase perform many functions:

- One of its main functions is to regulate the number and kind of RNA transcripts formed in response to the cell's requirements.



- Search and binds to promoter site.
- Unwind a short stretch of double helical DNA.
- Selects correct ribonucleotide and catalyze the formation of phosphodiester bond.
- Detect termination signals.
- Interact with terminator and regulator proteins that regulates rate of transcription.

### ❖ Mechanism of transcription:

Transcription starts at RNA polymerase site called **promoter** on template strand.

- In **prokaryotes** there are two binding sites **TTGACA** also called **-35** sequence and **TATAAT** sequence also called as **-10** sequence which have affinity for RNA polymerase.
- In **eukaryotes** these sites are at **-75** and **-25** respectively.

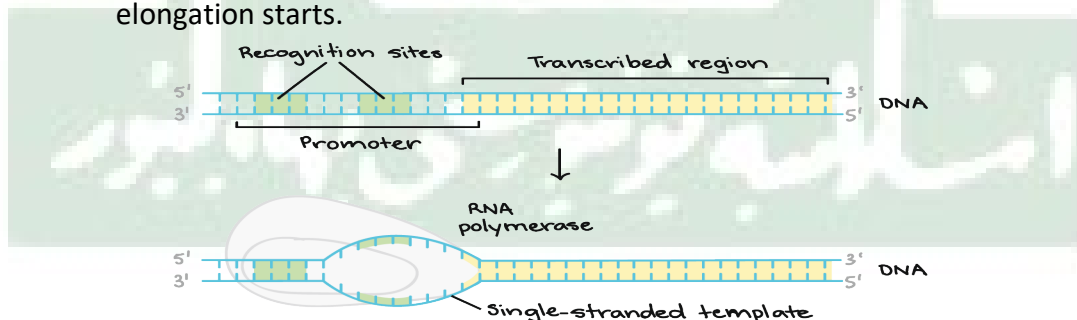
### ❖ Steps of transcription:

There are following steps of transcription:

#### 1. Initiation:

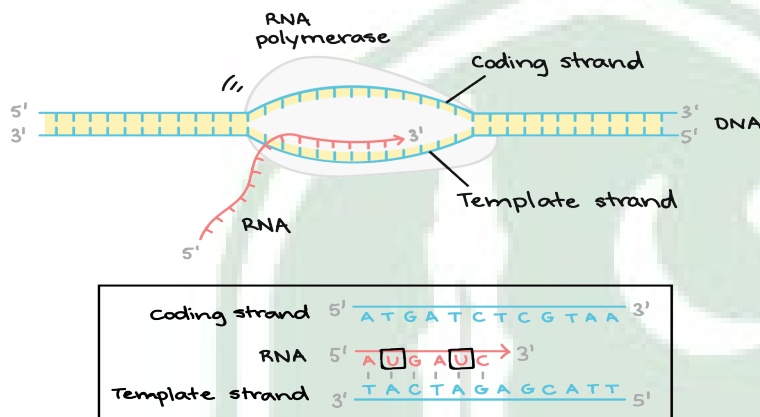
Following steps are involve in initiation of transcription:

- The site of DNA where RNA polymerase attaches is called as **promoter**. Promoter is normally composed of **50** nucleotides. It is present at the start of gene. The promoter for polymerase contains a **TATA** box. The TATA box binding protein (TBP) recognizes TATA boxes and attached the RNA polymerase on promoter.
- At this stage, DNA is double stranded. This RNA polymerase /wound -DNA structure is called **closed complex**.
- The DNA is unwound and become single stranded near initiation site. This RNA polymerase /unwounded structure is called **open complex**.
- The RNA polymerase transcribes the DNA, but produces about 10 abortive nucleotides. These are unable to leave RNA polymerase because exit channel is blocked by sigma factor.
- The sigma factor of RNA polymerase dissociates from holoenzyme and elongation starts.



## 2. Elongation:

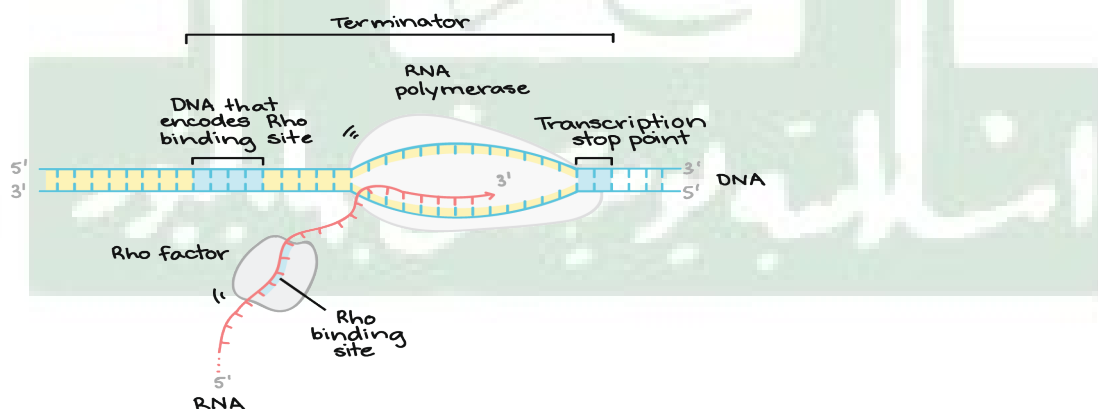
During elongation, RNA polymerase "walks" along one strand of DNA, known as the template strand, in the 3' to 5' direction. For each nucleotide in the template, RNA polymerase adds a matching (complementary) RNA nucleotide to the 3' end of the RNA strand. But in RNA, the base uracil replaces the base thymine. Thus, uracil complements to adenine.



## 2. Termination:

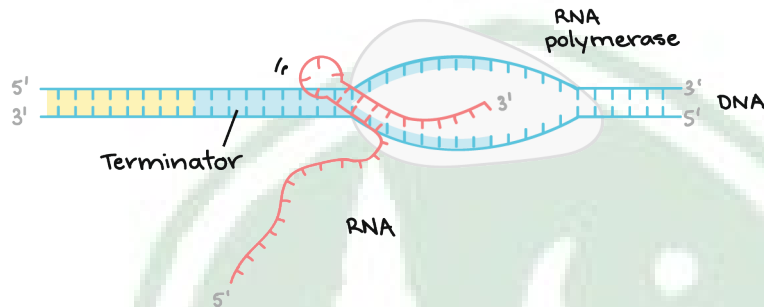
Transcription continues. Finally mRNA reaches termination sequences. There are two types of mechanisms of termination:

- In **Rho-dependent termination**, the RNA contains a binding site for a protein called Rho factor ( $\rho$  factor). Rho factor binds to this sequence and starts "climbing" up the transcript towards RNA polymerase. Rho factor dissociates RNA polymerase from DNA, terminating transcription.



- **Rho-independent termination** depends on specific sequences in the DNA template strand. As the RNA polymerase approaches the end of the gene being transcribed, it hits a region rich in C and G nucleotides. The RNA transcribed from this region folds back on

itself, and the complementary C and G nucleotides bind together. The result is a stable hairpin that causes the polymerase to stall. In a terminator, the hairpin is followed by a stretch of U nucleotides in the RNA, which match up with A nucleotides in the template DNA. The complementary U-A region of the RNA transcript forms only a weak interaction with the template DNA. This, coupled with the stalled polymerase, produces enough instability for the enzyme to fall off and liberate the new RNA transcript.



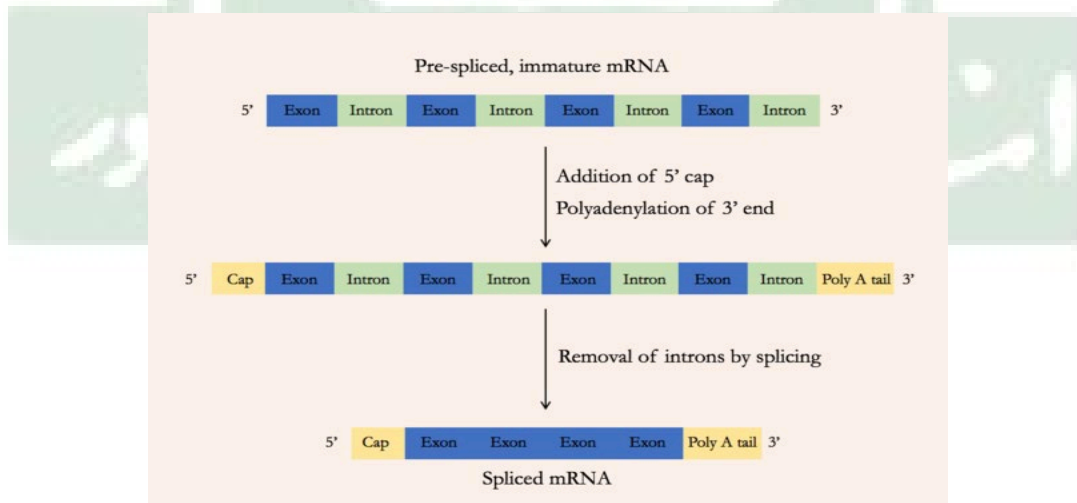
## **Post transcriptional modification:**

### **❖ Definition:**

Post transcriptional modification is a process in which primary transcript RNA is converted into mature RNA.

### **❖ Explanation:**

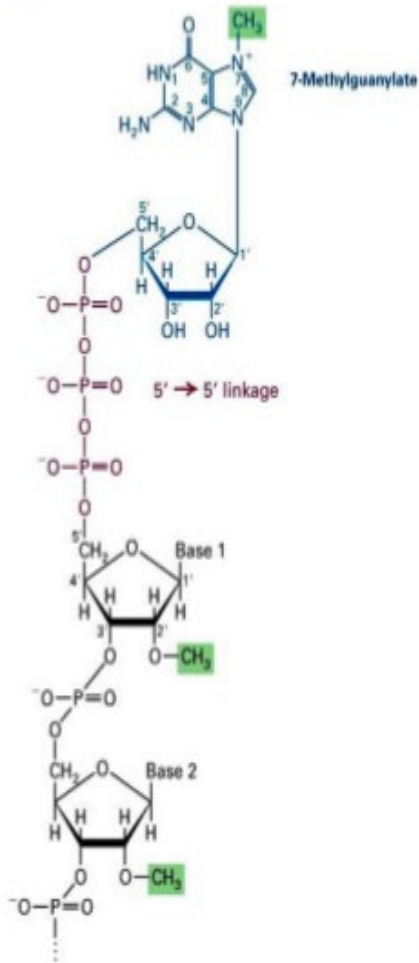
During the pre-mRNA processing phase, many nuclear proteins are recruited to the mRNA being processed to form RNA-protein complexes, and only a successfully processed mRNA will form a proper mRNA–protein complex, which is required to pass through the nuclear pore complexes. Therefore, defective mRNA and other byproduct RNA molecules, such as spliced-out introns, will be subjected to degradation by a multi-subunit exosome complex specialized for RNA degradation



## ❖ Process of post transcriptional modifications:

Post transcriptional modification includes:

- Capping
- Tailing
- Splicing
- Micro RNA



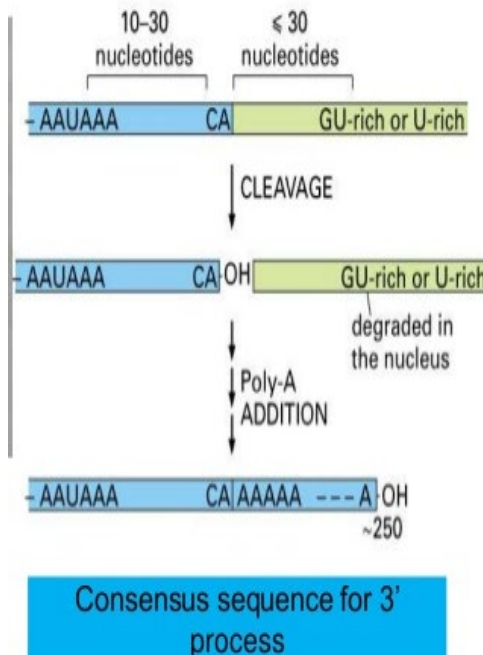
### • Capping:

- Capping of the pre-mRNA involves the addition of 7-methylguanosine ( $m^7G$ ) to the 5' end.
- Cap is added after first 20-30 nucleotides of mRNA have been synthesized.
- Formation of cap requires three steps
  - The terminal 5' phosphate requires removal, which is done with the aid of a phosphatase enzyme. The enzyme guanosyl transferase then catalyzes the reaction, which produces the diphosphate 5' end.
  - The diphosphate 5' end then attacks the alpha phosphorus atom of a GTP molecule in order to add the guanine residue in a 5'5' triphosphate link.
  - The enzyme (guanine- $N^7$ )-methyltransferase ("cap MTase") transfers a methyl group from S-adenosyl methionine to the guanine ring. This type of cap, with just the ( $m^7G$ ) in position is called a **cap 0** structure.

### ➤ Cap functions:

Cap provides:

- Stability to mRNA
- Enhanced translation and splicing
- Protection from ribonuclease degradation
- Enhanced transport from nucleus to cytoplasm

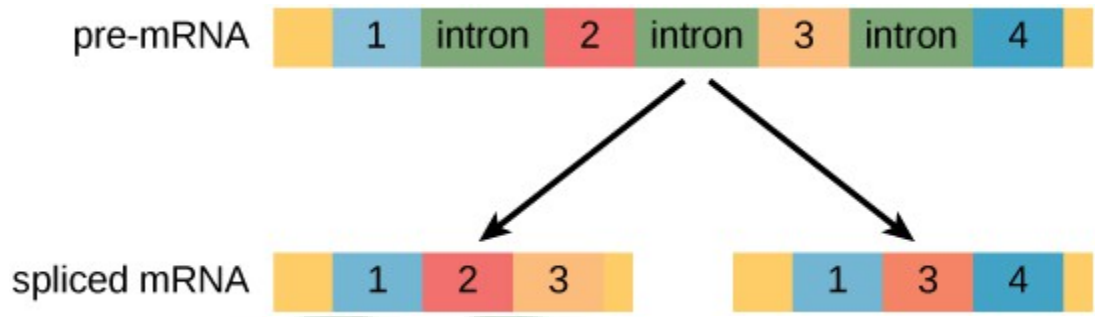


## ● Polyadenylation:

- The pre-mRNA processing at the 3' end of the RNA molecule involves cleavage of its 3' end.
- And then the addition of about 250 adenine residues to form a poly (A) tail.
- A **GU-rich sequence** is also usually present further downstream on the pre-mRNA molecule.
- Adenine nucleotides are added by enzyme **adenyl transferase**.
- Two main steps are required:
  - Cleavage of mRNA at **3'** end
  - Addition of adenine residue

## ● m RNA splicing:

- Non coding sequences are called **introns** and coding sequences are called **exons**.
- Removal of introns from primary mRNA transcript is called as RNA splicing.
- Introns are removed from the pre-mRNA and the remaining exons connected to re-form a single continuous molecule.
- Although most RNA splicing occurs after the complete synthesis and end-capping of the pre-mRNA, transcripts with many exons can be spliced co-transcriptionally.
- The splicing reaction is catalyzed by a large protein complex called the **spliceosome** assembled from proteins and **small nuclear RNA** molecules that recognize **splice sites** in the pre-mRNA sequence.
- . Many pre-mRNAs, including those encoding **antibodies**, can be spliced in multiple ways to produce different mature mRNAs that encode different **protein sequences**.
- This process is also known as **alternative splicing**.
- Processing of core histones is done differently because typical histone mRNA lacks several features of other eukaryotic mRNAs, such as poly (A) tail and introns. Thus, such mRNAs do not undergo splicing and their 3' processing is done independent of most cleavage and polyadenylation factors. Core histone mRNAs have a special **stem-loop** structure at 3-prime end that is recognized by a **stem-loop binding protein**. This is called **histone mRNA processing**.



### • Micro RNAs:

- Micro RNAs (miRNA) are among first small regulatory RNAs to be discovered.
- A miRNA is first transcribed as a long RNA molecule, which forms base pairs with itself and folds over to make a hairpin.
- Hair pin is capped by enzymes, releasing a small double stranded fragment of about **22** nucleotides.
- One of strands in this fragment is mature miRNA which binds to specific protein to make RNA-protein complex.
- The miRNA directs the protein complex to matching mRNA molecules.
- If miRNA and its target match perfectly, an enzyme in RNA-protein complex will typically chop mRNA in half, leading to its breakdown.
- If miRNA and its target have some mismatches, then RNA-protein complex may instead bind to mRNA and keep it from being translated.

