بسم الله الرحمـٰن الرحيم

(وَفَوْقَ كُلِّ ذِي عِلْمٍ عَلِيمٌ)

Cytology & Molecular Biology | FINAL 15

Chromatin Immunoprecipitation & Eukaryotic mRNA Processing (Transcription pt.3)

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وَلِلَّهِ الْأَسْمَاءُ الْحُسْنَى فَادْعُوهُ بِهَا

المعنى: واسع صفات الكمال ومتعلقاتها، العظيم وأفعاله عظيمة، واسع الكرم، مجّدَه خلقه لعظمته.

الورود: ورد مرتين في القرآن.

الشاهد: ﴿ إِنَّهُۥ حَمِيدٌ مَّجِيدٌ ﴾ [هود :٧٣].

٥٣

اضغط هنا لشرحٍ أكثر تفصيلًا

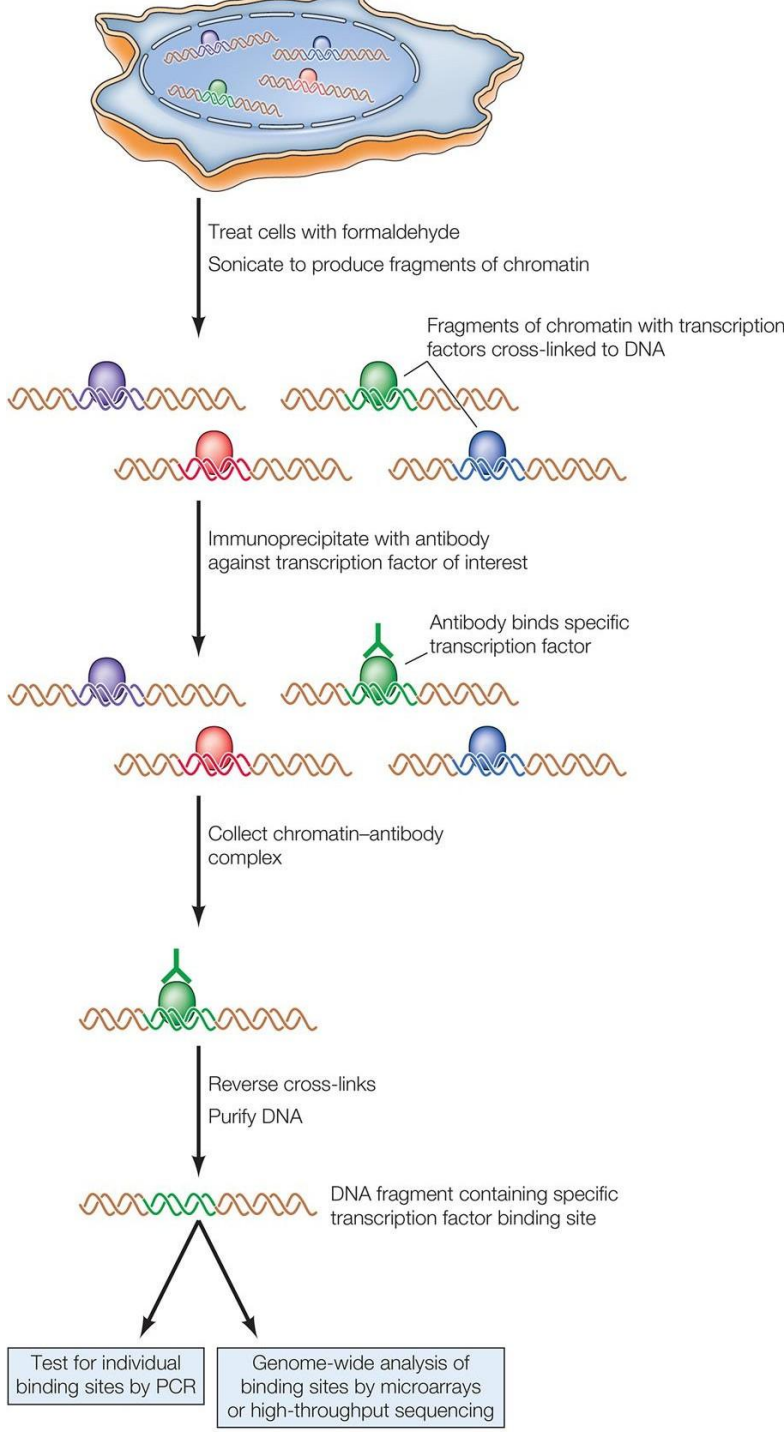
❑ Chromatin immunoprecipitation

(Chromatin = DNA + Protein) (“Immuno” = antibodies) (Precipitation = ترسبات)

❖ What is the purpose of this technique?

Transcription factor binding sites can be identified by chromatin immunoprecipitation.

- ➢ Proteins bound to DNA are chemically cross-linked to the DNA regions they are bound to.
  - Transcription factors (e.g., androgen receptor) bind DNA non-covalently and reversibly, so cross-linking converts this interaction into a covalent, stable bond
- ➢ DNA is isolated and fragmented.
  - Cells are first exposed to the hormone, so the receptor binds its target sites in vivo. The chromatin is then isolated and sheared into small fragments (by sonication or enzymes), each fragment containing DNA plus any bound proteins.
- ➢ The fragments are “immunoprecipitated” with an antibody against a specific transcription factor.
- ➢ The cross-links are reversed, and the immunoprecipitated DNA fragments are analyzed by PCR to test for the presence of a specific DNA sequence or by next-generation DNA sequencing microarrays or microarrays to identify all the binding sites for the transcription factor within the genome.



❑ Quick Summarization:

- ❖ We are interested in identifying the specific DNA sequences that proteins (e.g., transcription factors) bind to.

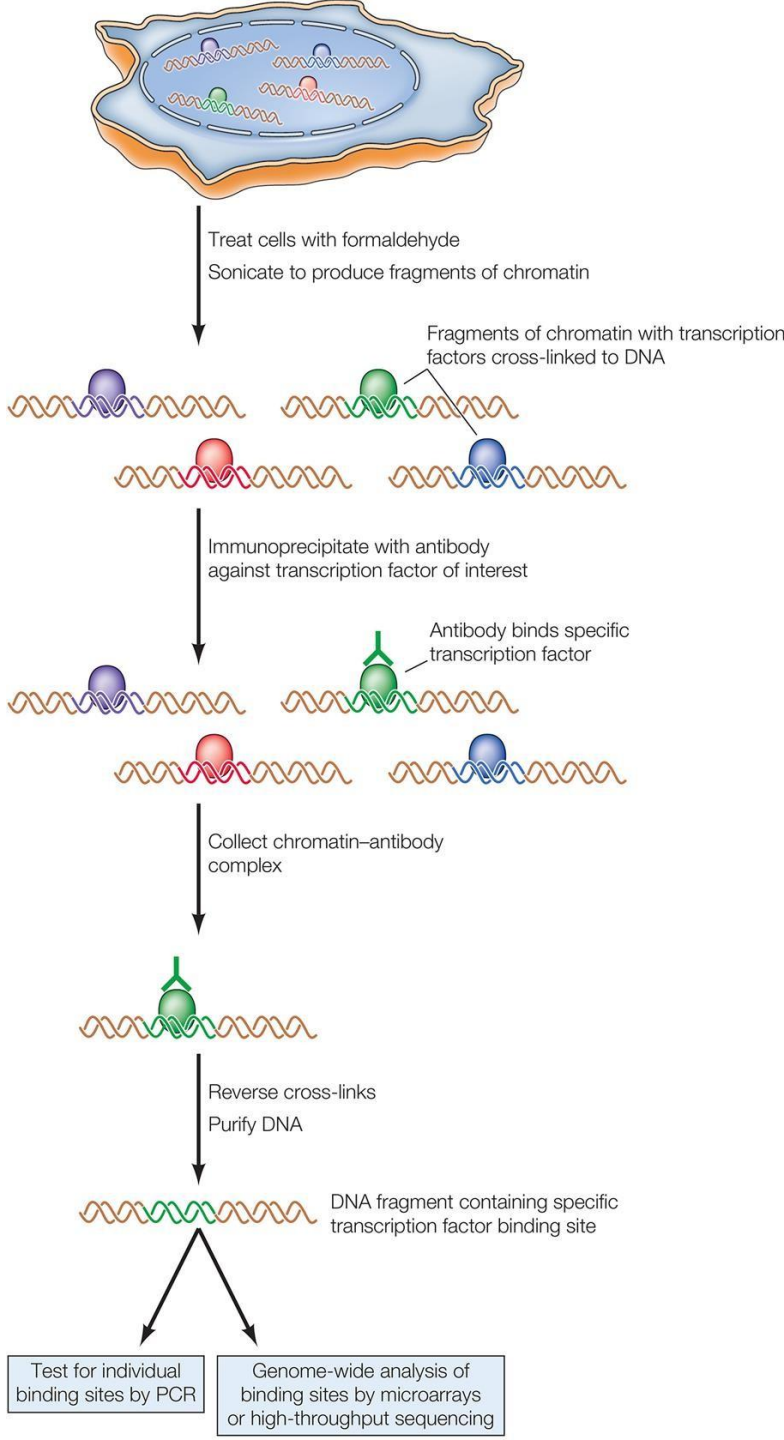
1. Cells are treated with cross-linking chemicals to covalently link proteins to DNA, stabilizing the normally non-covalent protein-DNA interactions.
2. DNA is then extracted and fragmented, usually at random, so each fragment contains DNA with any bound proteins.
3. Antibodies specific to the protein of interest are added, allowing immunoprecipitation of the protein together with its bound DNA fragment.
4. The cross-links are reversed, releasing and purifying the associated DNA.

- ▪ The recovered DNA fragments are analyzed using:

A. PCR (to test binding to a known sequence)
B. Next-generation DNA sequencing or microarrays (to identify genome-wide binding sites)

- ✓ Example:

Androgen binds to its receptor → receptor dimerizes → the dimer enters the nucleus (acts as a transcription factor) → binds androgen response elements → protein-DNA cross-linking → DNA fragmentation → antibody addition → immunoprecipitation → cross-link reversal → DNA analysis.

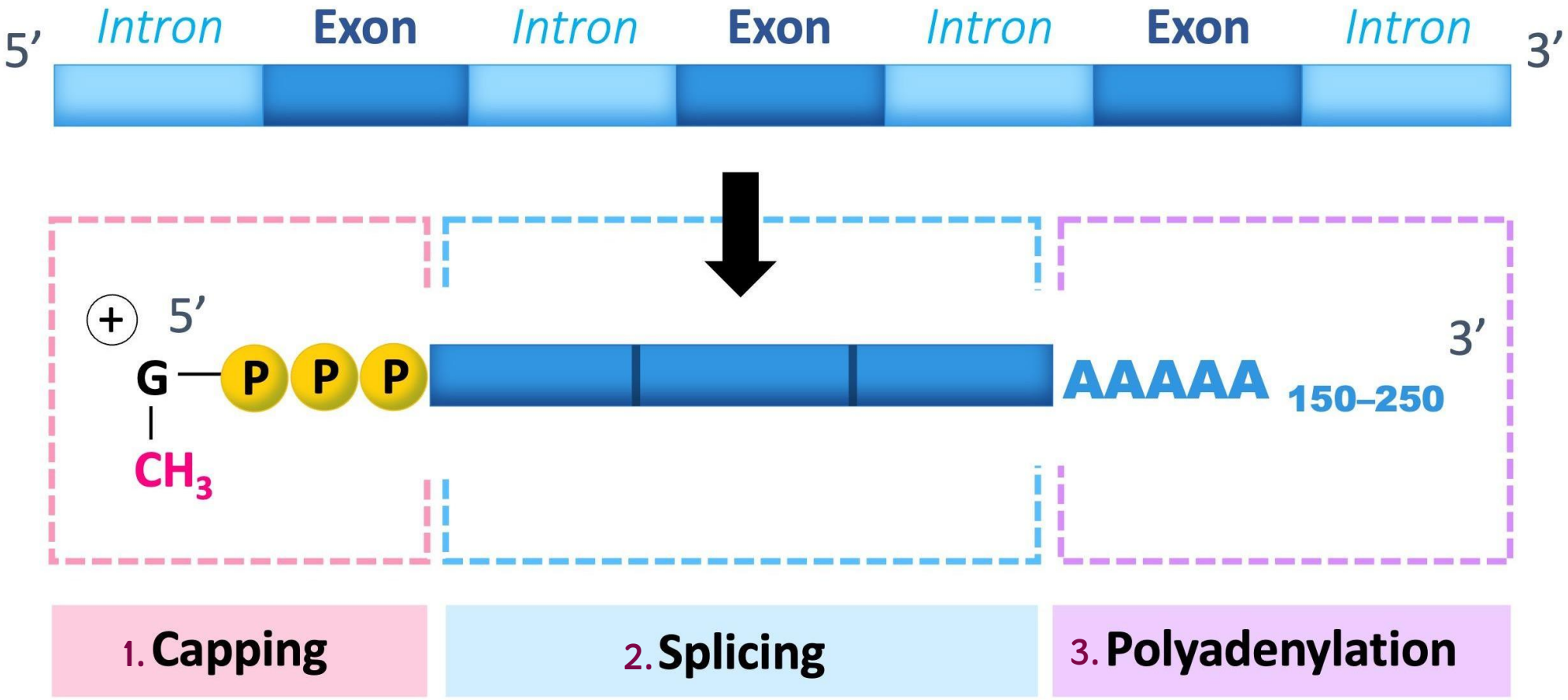


Eukaryotic RNA processing

Following Eukaryotic transcription

❑ Processing of mRNA in eukaryotes

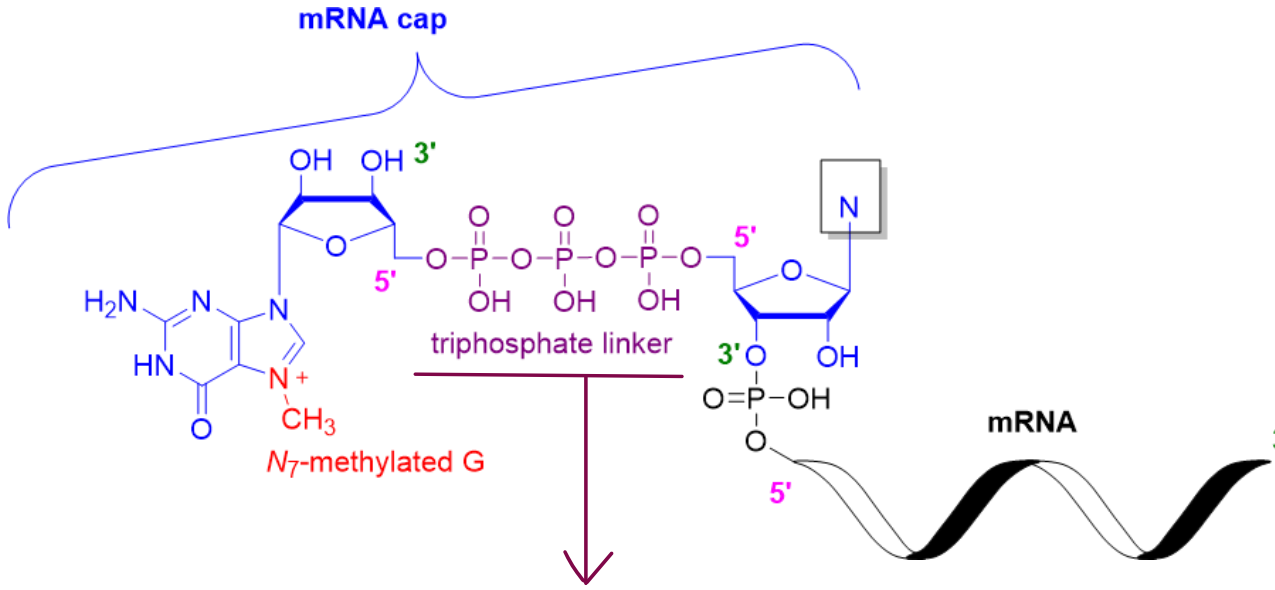
- In eukaryotic (human) cells, mRNA cannot be translated immediately after transcription; it must first undergo processing and modification. There are three main mRNA modifications:



1. Addition of a cap

(A cap is something at the beginning of molecule)

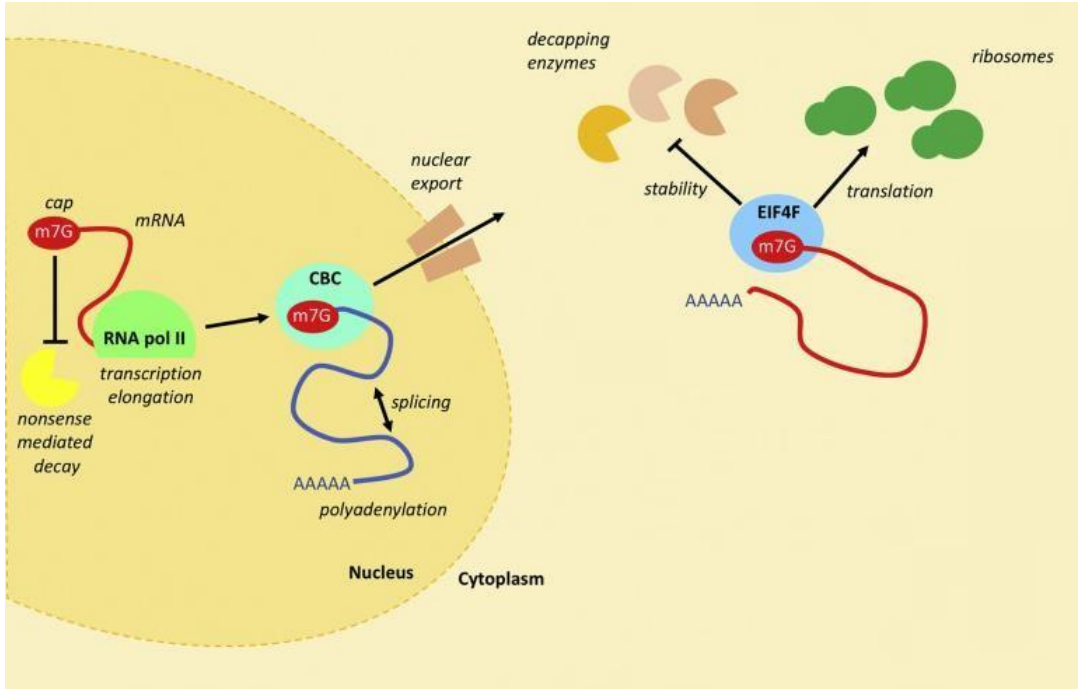
- The first modification comes as soon as RNA polymerase II has produced a few nucleotides of pre- mRNA.
- The 5' end of the new RNA molecule is modified by the addition of a "cap" that consists of a 7- methylguanosine molecule.



Note that the first nucleoside in the mRNA molecule have triphosphates, cause no breakage occur to be added to some nucleoside before it.

❖ Importance of capping

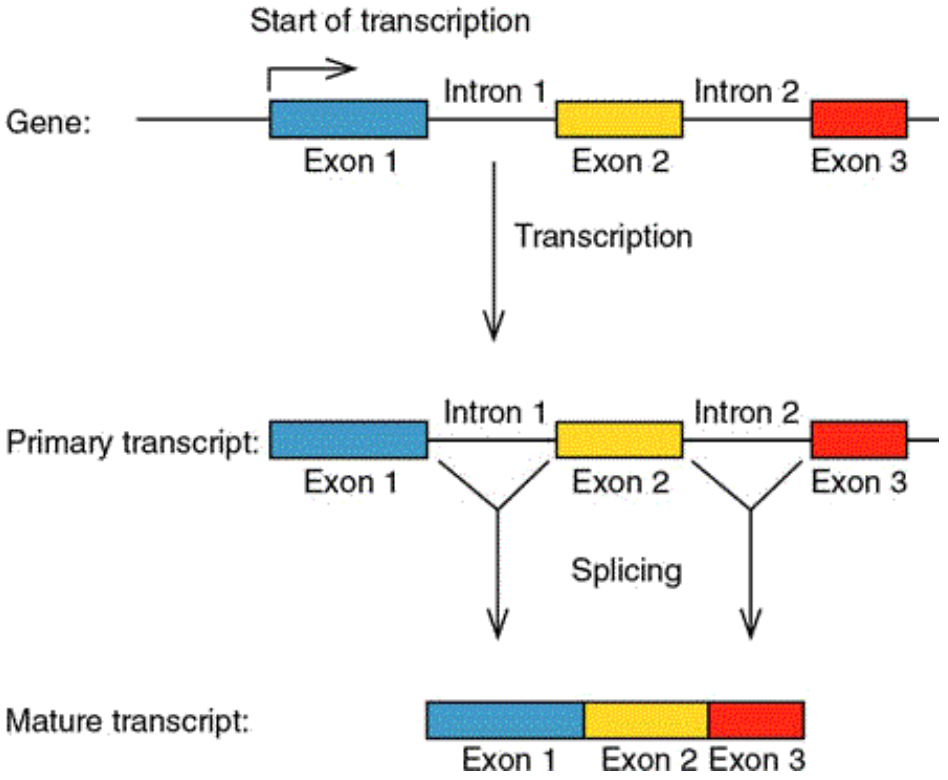
- It differentiates mRNA from other RNA molecules. Other RNAs are not capped
- It stabilizes the mRNA. So, it can't be degraded by nucleases
- It signals the 5' end of eukaryotic mRNAs.
- It recruits proteins necessary for splicing and polyadenylation.
- It helps in exporting RNA to the cytoplasm. Nuclear exporters recognize the cap then allow it to be exported.
- It helps in the translation of mRNAs to proteins. Since it marks 5' end.



2. Introns vs. exons and RNA splicing (splicing = cutting)

- The protein-coding genes of eukaryotic cells contain specific DNA sequences known as introns, which are transcribed but not translated.
- The protein-coding regions are known as exons.
- When RNA is synthesized, the RNA molecule contains both introns and exons and is known as primary transcript or pre-mRNA.
- The intron sequences are removed from the newly synthesized RNA through the process of RNA splicing, and exons are connected
- Now the RNA molecule is known as mRNA (mature transcript) or mature mRNA

mRNA is made as primary transcript

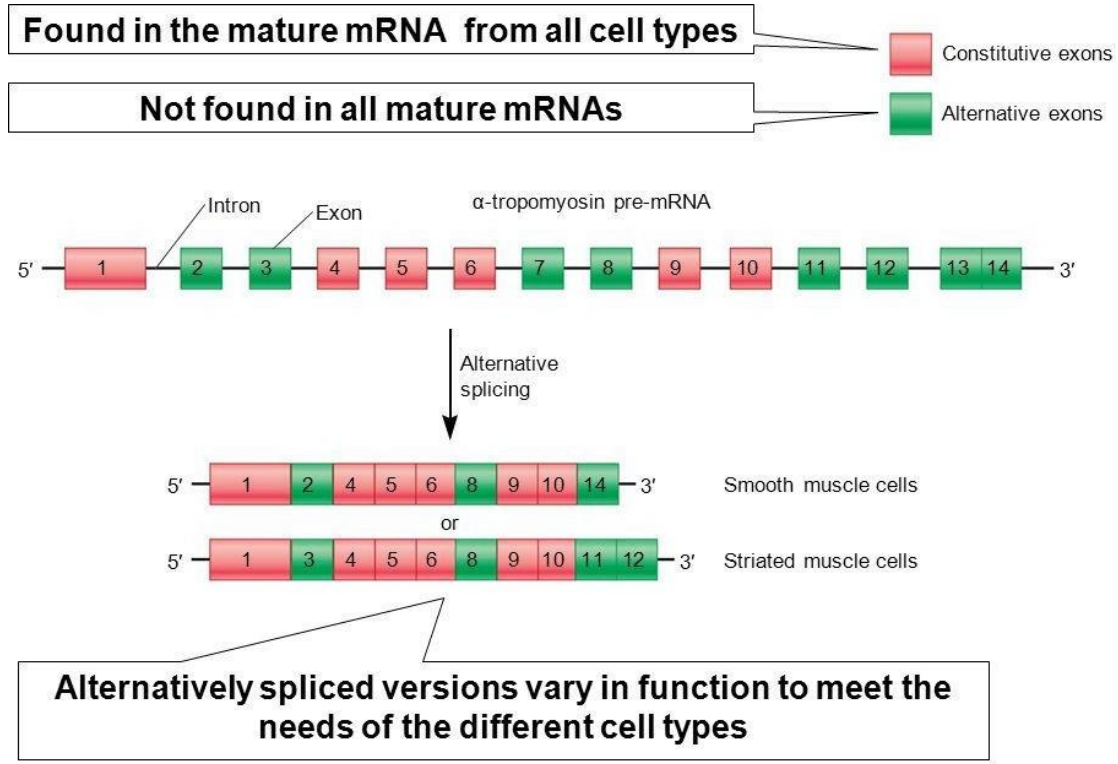


❖ Alternative splicing for mRNA

- The transcripts are spliced in different ways to produce different mRNAs and different proteins (known as protein isoforms, which are highly related gene products that perform essentially the same biological function).
- A classic example is tropomyosin, an actin-binding protein, The same gene can undergo different splicing patterns in different cell types, producing multiple mRNA isoforms and therefore different protein variants.
- These variants interact differently with actin, contributing to cell-specific structure and function of the cytoskeleton.
- Alternative splicing allows organisms to generate a wide diversity of proteins from a limited number of genes, explaining how gene number does not directly reflect protein complexity.

Note: Exons that are 3' to another exon are never placed 5' to it after splicing.

(Which means order is preserved)



Few notes:

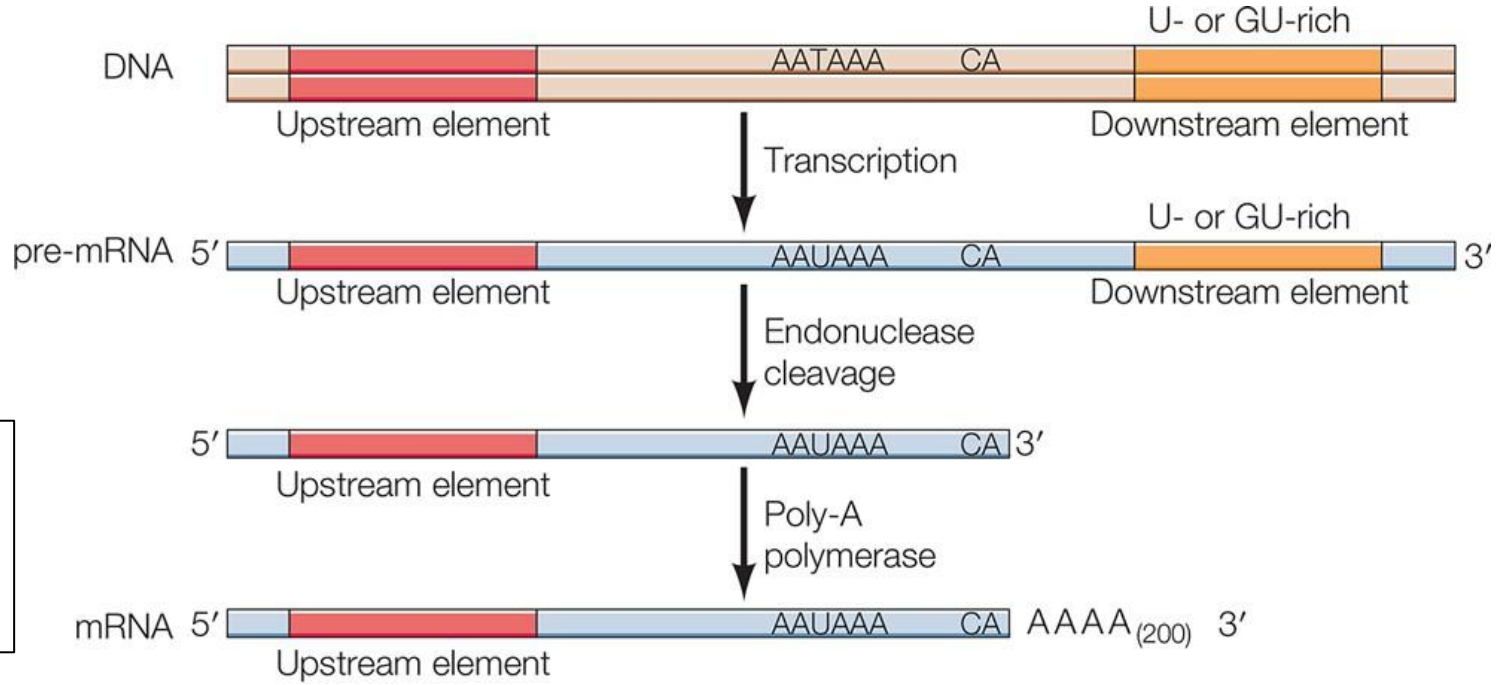
- Constitutive exons: are always found in the transcript of a certain protein (gives the identity to a protein)
- Alternative exons: responsible for isoforms

3. Polyadenylation (addition of nucleotide A)

- A certain sequence in the mRNA (AAUAAA) (Followed by CA) signals the end of transcription and it is part of the 3' ends of mRNAs. It is AATAAA on the coding/ sense/ non-template strand of DNA in genome
- The pre-mRNA cleaved after this sequence. It is a signal for termination
- Poly-A polymerase then adds ~200 A nucleotides to the 3' end.
- The nucleotide precursor for these additions is ATP.

Why polyA?
1. mRNA transport from the nucleus to the cytosol.
2. It helps in translation.
3. It stabilizes mRNA.
   So, it dose not get degraded

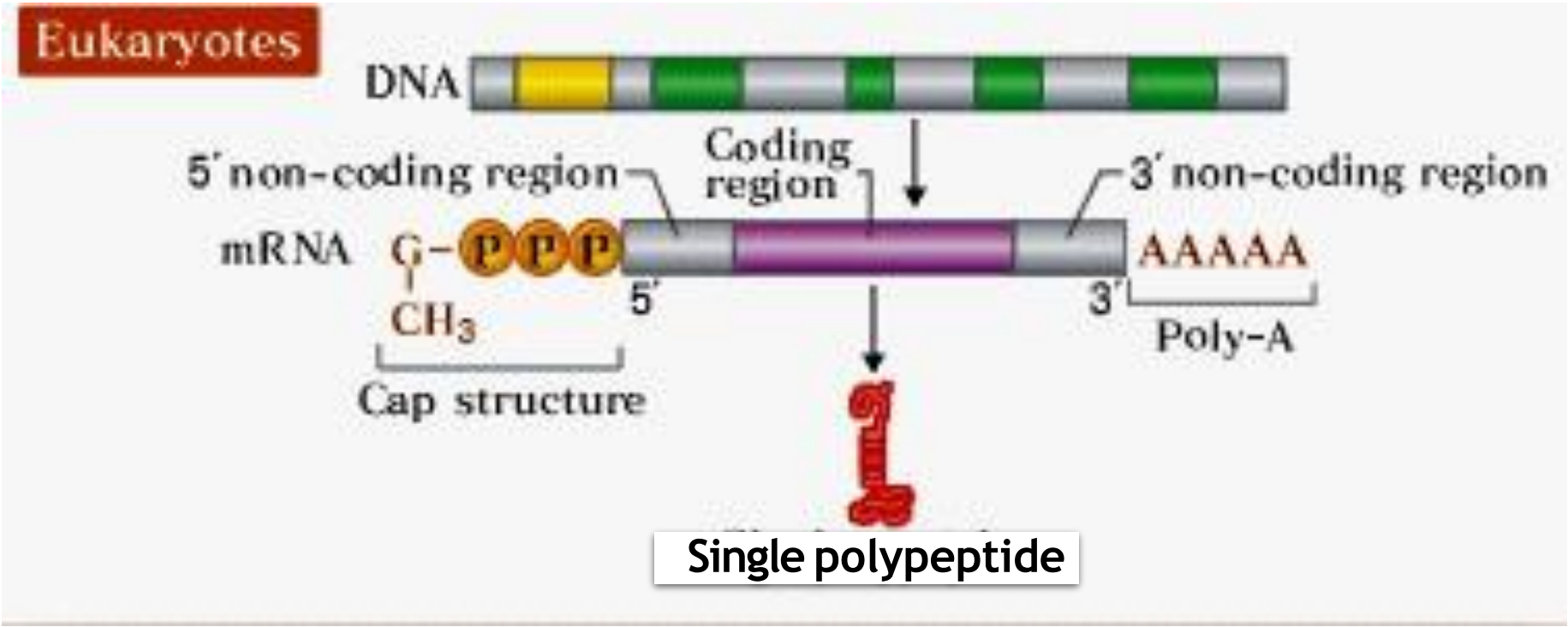
Poly-A polymerase does not require a template, and the poly-A tail is not encoded in the genome.
(It is not found in the gene; it is smth additional)



❑ Eukaryotic genes

- Eukaryotic transcription units produce mRNAs that encode only one Protein, thus termed monocistronic, This polypeptide can be processed in different ways

Unlike prokaryotic cistron that can be polycistronic



❑mRNA transport

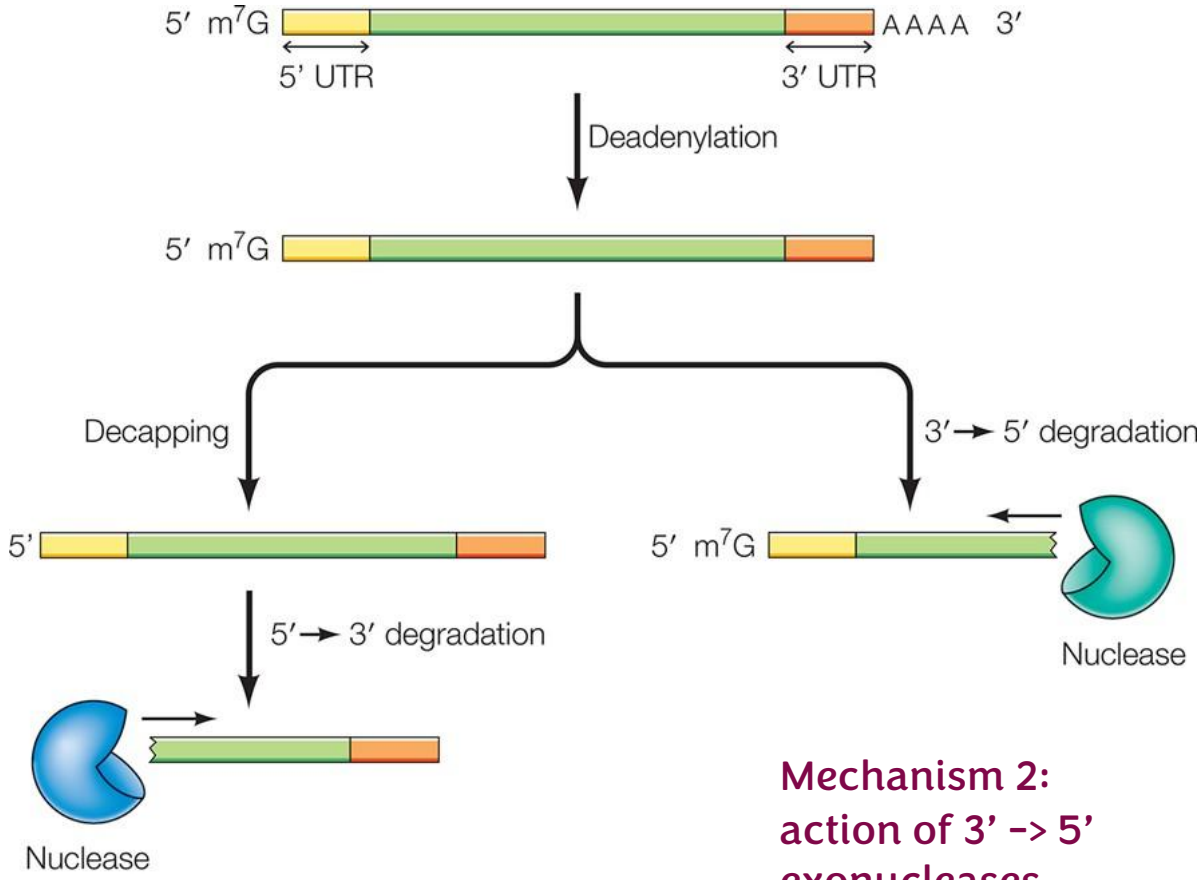
- Transport of mRNA from the nucleus to the cytoplasm, where it is translated into protein, is highly selective- and is associated to correct RNA processing.

- Defective mRNA molecules like interrupted RNA, not capped, poly A tailed, mRNA with inaccurate splicing, very long mRNA (in case AATAAA signal is removed from the gene) , and so on, are not transported outside the nucleus.

❑ Degradation of mRNAs

- The half-lives of bacterial mRNA is about 3 minutes.
- The half-lives of eukaryotic mRNAs can be on average 30 minutes but can be longer.
- Degradation of eukaryotic mRNA is initiated by shortening of poly-A tail followed by action of 3'-to-5' exonucleases or decapping (removal of cap) and then 5'-to-3' exonucleases.

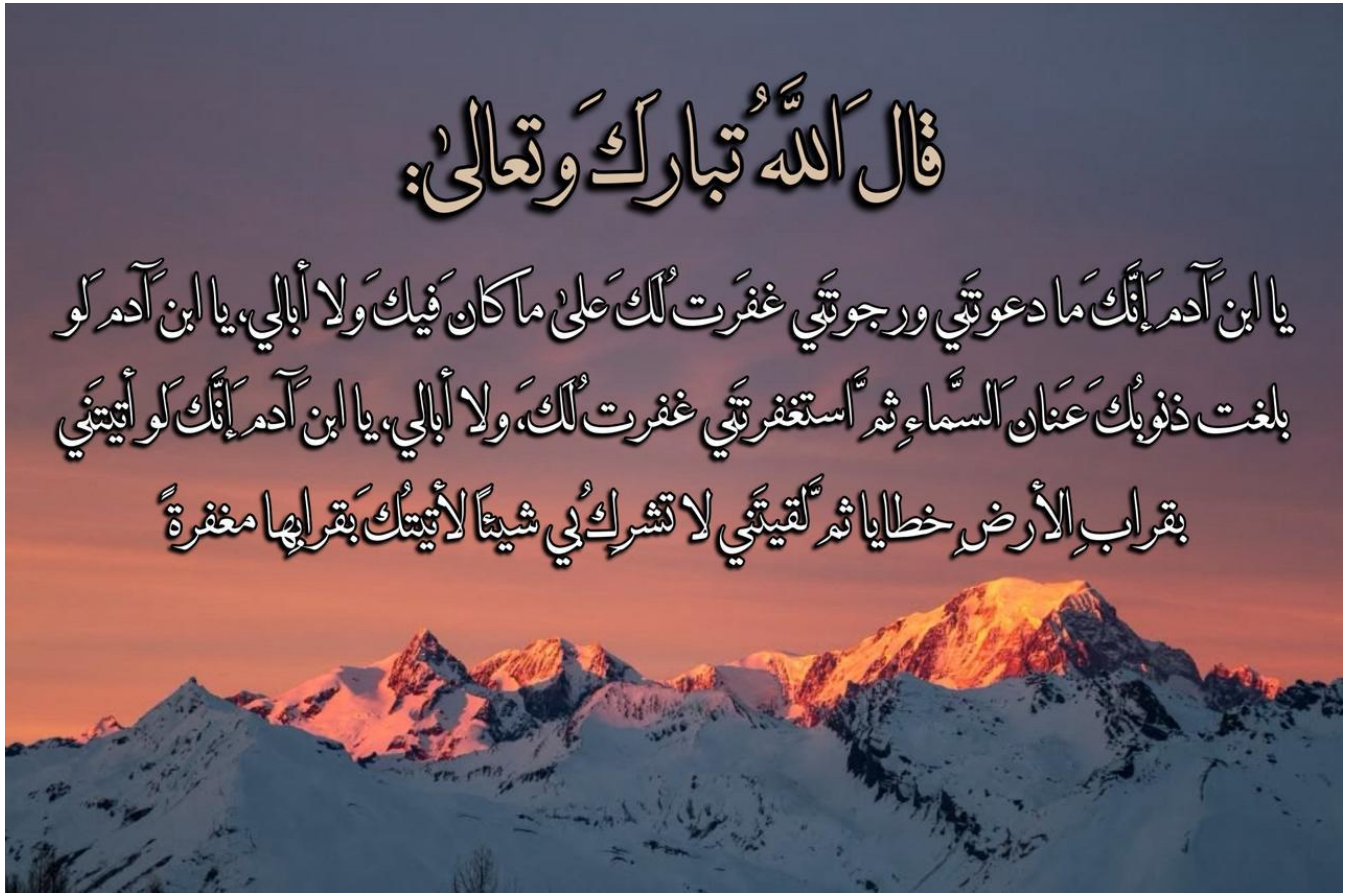
As the stability of mRNA increases→ its half-life increases→ stay more in cytosol → more coding for the protein→ high protein levels



Mechanism 1:
decapping followed by
5' -> 3' exonucleases action

Mechanism 2:
action of 3' -> 5'
exonucleases

رسالة من الفريق العلمي:



اللهُ عزَّ وجلَّ هو أرحمُ الرَّاحِمين, يغْفِرُ الذُّنوبَ للعاصِي والمذْنبِ منْ عبادِه مهما بلَغَت, لكنْ بشرطِ أنْ يكونَ هذا العبْدُ مُوحِّدًا لربِّه لا يُشْرِكُ به شيئًا, فكلُّ ذنْبٍ تحْتَ مشيئةِ اللهِ تعالى فيغْفِرُ لِمنْ يشاءُ, إلَّا الشِّركَ, فإنَّ اللهَ تعالى لا يَغْفِرُه إذا ماتَ الإنسانُ عليه ولَقِيَه به.

وفي الحديثِ: فضلُ التَّوحيدِ, وأنَّ اللهَ يَغفِرُ للمُوحِّدين الذُّنوب والمعاصى.
وفيه: سَعَةُ رحمةِ اللهِ تَعالى ومغفرتِه وفَضلِه.
وفيه: خُطورةُ الشِّركِ والتَّحذيرُ منه.

الشرح الكامل للحديث

For any feedback, scan the code or click on it.

Corrections from previous versions:

Versions	Slide # and Place of Error	Before Correction	After Correction
V0 → V1			
V1 → V2			