11

Molecular Biology

Doctor 2019 | Medicine | JU

◉ Sheet ○ Slides

Done By

Bushra A. AlQudhah

Contributed in the Scientific Correction

Abdulrahman AlQudhah

Contributed in the Grammatical Correction

Abdulrahman AlQudhah

Doctor

Dr. Walhan Alshaer

Before we start:

Dr. Walhan put this question for you to make sure you get the principle of Transcription, please solve it:

If you have the following RNA sequence:

5'- AAUCCGUCGGGCCUCGUUC -3'

What is the sequence in DNA? Try to write it with the correct directions.

A Quick Review:

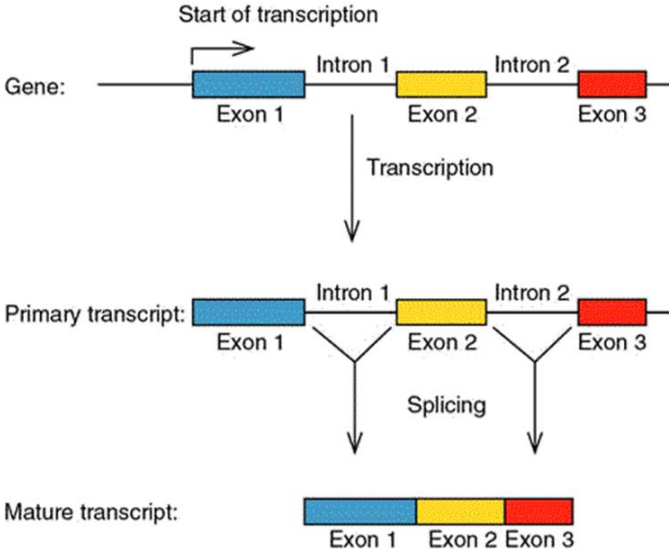
- Transcription in the Eukaryotes produces Pre-mRNA that contains both introns and exons.

- Introns are non-coding RNA that should be removed from the pre-mRNA to produce the mature RNA.

- Exons are the code for protein synthesis.

RNA Splicing:

The intron sequences are removed from the newly synthesized RNA through the process of RNA splicing.



Now the RNA molecule is known as mRNA (mature transcript).

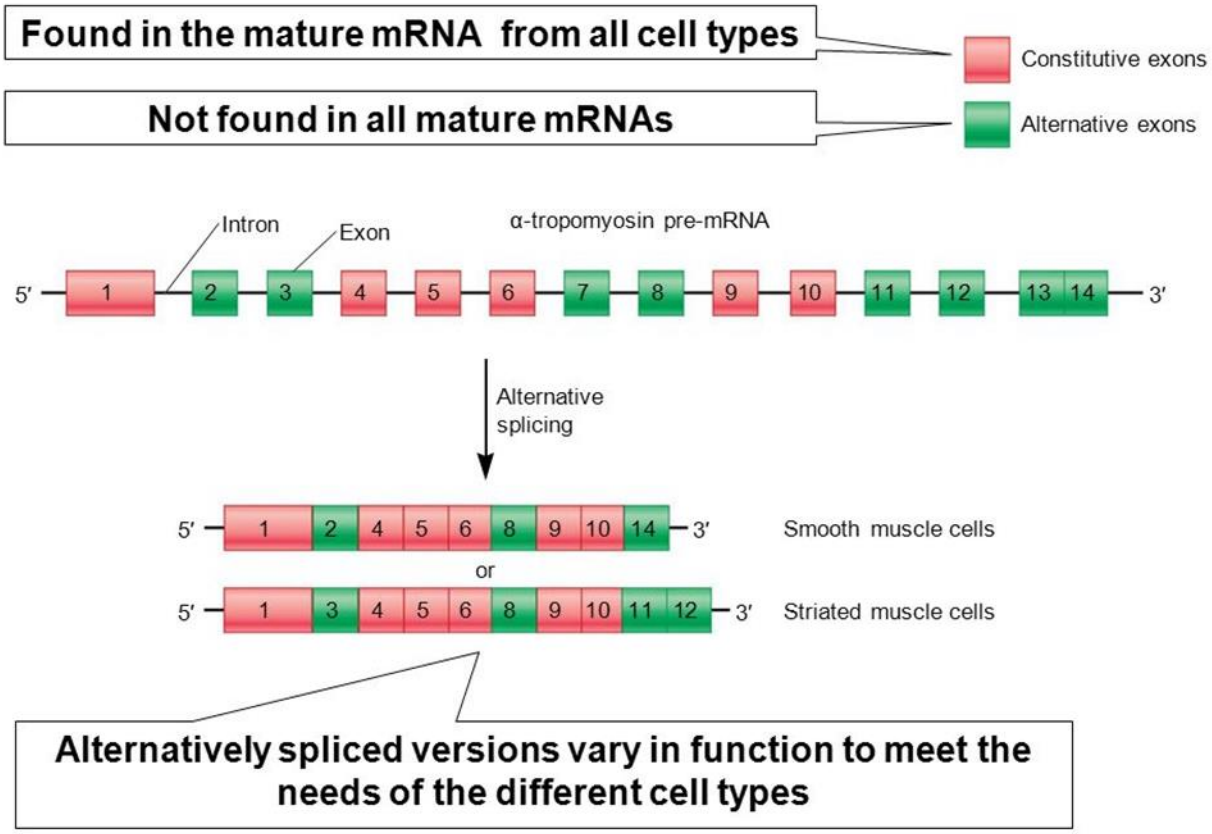
*But is RNA splicing going the same way all the time and producing one type of proteins?

RNA can sometimes be spliced in different ways; to produce different mRNA's and – therefore – different proteins.

Alternative Splicing:

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).

An example of this mechanism is α-Tropomyosin (protein involved mainly in muscle contraction and exists in muscle and brain cells and fibroblasts).
In the figure below:



This figure shows that:
In smooth and striated muscle cells, we have two different isoforms of α-tropomyosin.
These isoforms are formed as results of the same pre-mRNA that has been alternatively spliced to form these two different isoforms.

Note that the exon codes in the α-tropomyosin of the smooth muscle cells are (1,2,4,5,6,8,9,10,14)
But they are not the same for the protein isoform in striated muscle cells (1,3,4,5,6,8,9,10,11,12).
In both cells, the 2 isoforms of α-tropomyosin do the same function (muscle contraction). But we can see that we have different codes of exons in each isoform, and these different codes are a result of alternative splicing.
There are alternative exons that vary between isoforms (each isoform has some of them but not all of them) (green).
We also have constitutive exons; these ones are fixed and exist in all isoforms (red).

*What about exon no. 8 ? it exists in both cells when it is an alternative exon ?
It happened that these isoforms share this exon, but other isoforms of α-tropomyosin don't necessarily have it. (it's just a coincidence).

In conclusion:
Alternative splicing produces different mRNA's, and different mRNA's produce different isoforms that have the same biological function in different cells.

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

Processing of mRNA in eukaryotes:

We said in the previous lectures that transcription in Eukaryotes is more complicated than in Prokaryotes, and that it is highly regulated, which means that there are many processes for mRNA in Eukaryotes before it becomes ready for protein translation.
So, mRNA is processed and modified extensively in order to prepare it for protein translation, these processes include:

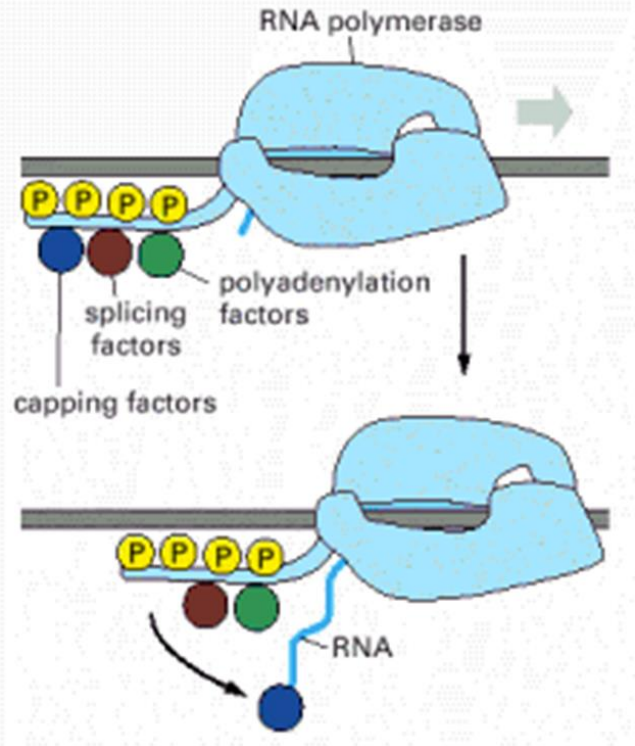
1. Capping: The addition of a cap on the 5' end of mRNA.

2. Splicing: The removal of introns and keeping the exons.

3. Polyadenylation: The addition of (A) nucleotides to the 3' end of mRNA.

These processes require proteins and enzymes, Some of these processing proteins are associated with the tail of RNA polymerase II.

These proteins jump from the polymerase tail onto the RNA molecule as it appears. (figure:)



Remember: The carboxyl terminus of the RNA Polymerase II (tail) is phosphorylated by TFIIH.

While the RNA is being synthesized, these proteins jump to the RNA to start doing their function. After synthesis of the first nucleotides, capping factors will jump to the RNA and start the capping process.

For polyadenylation, there are enzymes that jump to the tail of mRNA and start the polyadenylation process through the addition of adenine nucleotides to the tail of mRNA once the synthesis reaches the end of mRNA.

1.Addition of a Cap:

As soon as RNA polymerase II has produced about ~25 nucleotides of pre-mRNA, the 5' end of the new RNA molecule is modified by addition of a "cap" that consists of GTP, and this process is performed in reverse orientation.

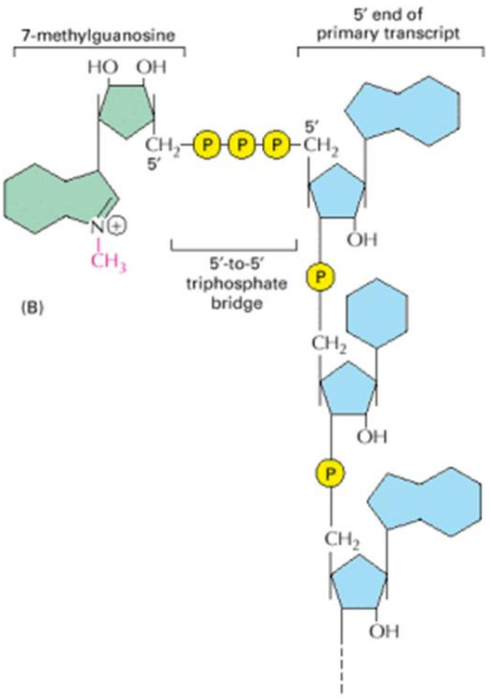
5’to 5’ instead of 5’ to 3 ’.

Extra explanation:

In DNA and RNA synthesis, the pentose sugars are connected on each strand via phosphodiester bonds formed between carbon no. 5’ of a sugar and carbon no. 3’ of the next sugar (whether they were ribose or deoxyribose) so we say: the orientation is 5’ to 3’.

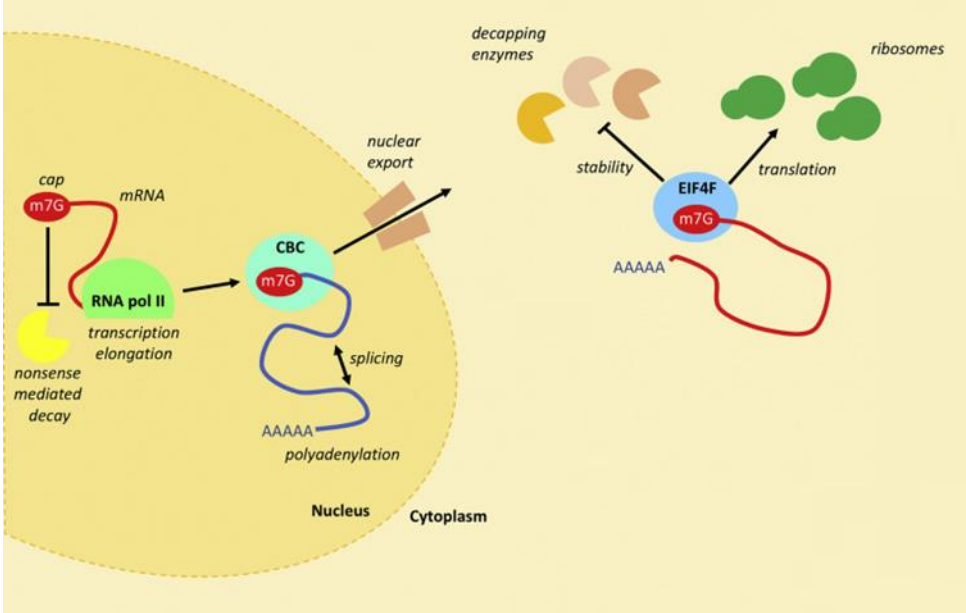
But in the case of Capping, the GTP is connected to the sugar via a bond that is laid between carbon no. 5’ in the GTP and carbon no. 5’ in the **ribose** sugar. So, we say: the orientation is 5’ to 5’.

هاد الكلام زيادة مني بس مشان توصلكم الفكرة



Importance of Capping:

- It stabilizes the mRNA:
  mRNA half-life is short, and we need it to be stable for the process of protein translation and to go through the processes of transcription.
- It signals the 5’ end of eukaryotic mRNA’s:
  This helps the cell to distinguish (identify) mRNAs from the other types of RNA molecules, which are uncapped.
- It recruits proteins necessary for splicing and polyadenylation.
- It helps in exporting RNA to the cytoplasm.
  Replication and Transcription occur in the nucleus in Eukaryotes, and the processing of pre-mRNA into mature RNA also occurs in the nucleus.
  Mature RNA should be exported into Cytoplasm where translation occurs. (figure)
- It helps in the translation of mRNAs to proteins.
  Explained in the previous point.



2.Polyadenylation:

Remember: In the termination phase of transcription mechanism in Eukaryotes, there is a signal that tells RNA Polymerase to stop the synthesis of RNA, and it consists of a sequence of AAUAAA in the mRNA followed by a G-U rich region.

In Details:

A certain sequence in the pre-mRNA (AAUAAA) in the 3' ends of it is recognized by enzymes that cleave it.

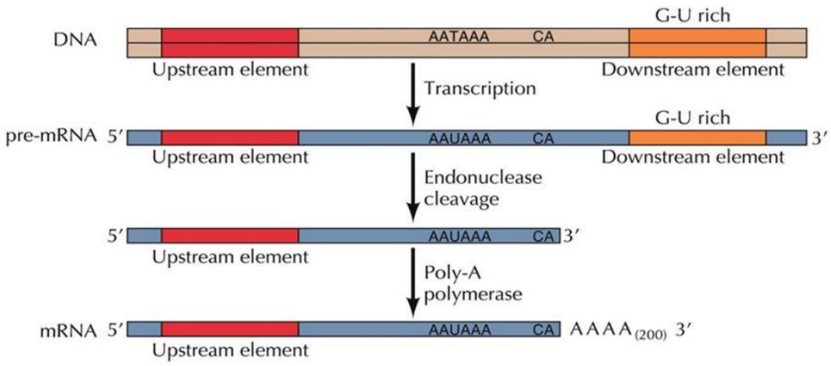
(to cleave mRNA at CA nucleotides at the 3' end)

The enzyme here is Endonuclease. Recall the difference between Endonuclease and Exonuclease enzymes:

Endonuclease: cleaves from the inside of the nucleic acid sequence.

Exonuclease: cleaves from an end (5' or 3').

So the endonuclease cleaves the pre-mRNA 10 – 30 nucleotides downstream of the (AAUAAA) at the CA sequence.



Poly-A polymerase then adds ~200 A nucleotides to the 3' end.

Note: Poly-A polymerase does not require a template and the poly-A tail is not encoded in the genome.

The nucleotide precursor for these additions is ATP.

Note: Poly-A polymerase enzyme catches the ATP and removes 2 phosphate groups, keeping only one phosphate group on it.

and that's why the nucleotide precursor for these additions is ATP, but the added adenine nucleotides have only one phosphate group (AMP).

يعني بيشيل مجموعتين فوسفات وبيبقي وحدة وهاي بتكون مصدر طاقة اله.

Significance of polyadenylation:

- It helps in transporting mRNA from the nucleus to the cytosol.
- It helps in translation. (of proteins).
- It stabilizes mRNA.

mRNA Transport:

As we mentioned before, transcription occurs in the nucleus, as well as processing of pre-mRNA into mature RNA. But translation of RNA into proteins occurs in the cytoplasm.

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, mRNA with inaccurate splicing, and so on, are not transported outside the nucleus.

This means that if we have any mistake in the RNA, it will not be transported to the cytoplasm.

***But why is this important?**

Imagine that we have a partially-transcripted RNA, and that it was supposed to carry a gene consisting of 10 exons that code for a certain protein.

But if this mRNA is partially-transcripted, what it will carry is only 5 exons. If this mRNA was transported to the cytoplasm, it will result in the production of a short and incorrect protein, and this will affect the cell's function.

And that is why our cells have the capability of controlling the transport of the correct mRNA to the cytoplasm, allowing the synthesis of the correct proteins.

Degradation of mRNAs:

Remember: The significance of mutations in RNA are less bad than mutations in DNA; for if there is a mutation in mRNA, it is to be eliminated; because mRNA will be degraded later. But if there is a mutation in DNA, it will stay in our cells.

So, based on that, what will happen to mRNA after doing its job? Will it stay forever?

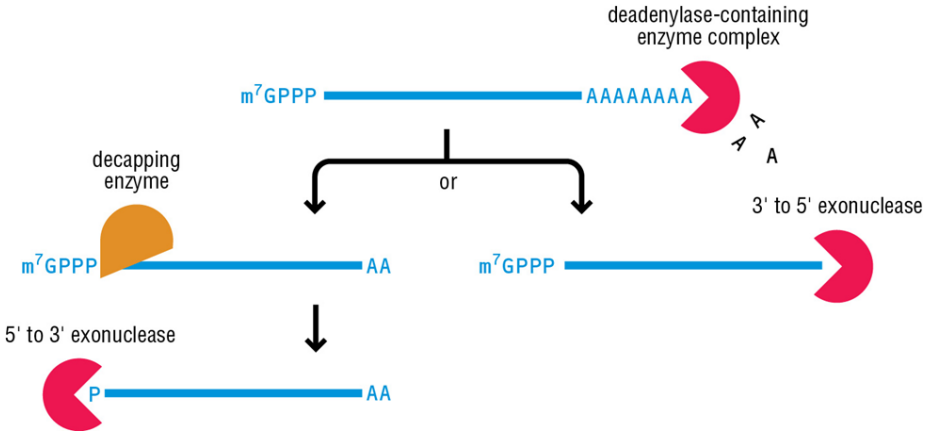
Absolutely not, in both Eukaryotes and Prokaryotes, mRNA will be degraded, but there are some differences between them:

- The vast majority of mRNAs in a bacterial cell are very unstable, having a half-life of about 3 minutes.
- The mRNAs in eukaryotic cells are more stable (up to 10 hours; average of 30 minutes).

***Then, how does degradation occur in mRNA?**

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.

(figure)



In this figure, the enzyme (deadenylase) is responsible of removing Adenine nucleotides from the 3' end of mRNA and by so, shortening it.

And then, the resulting strand gets cleaved by 3' to 5' exonuclease or gets decapped and then cleaved by 5' to 3' exonuclease.

Please note the directions of exonucleases in both cases.

DNA:TTTTTTTTT
RNA:


أنت الي مخلص شيت
1/2/3/4/5/6/7/8/9/10/11
كويزات ومحاضرات
مراكمة لمواد
ثانية