CYTOLOGY

Premed 2018 - JU

◉ Sheet ○ Slides

Number

14

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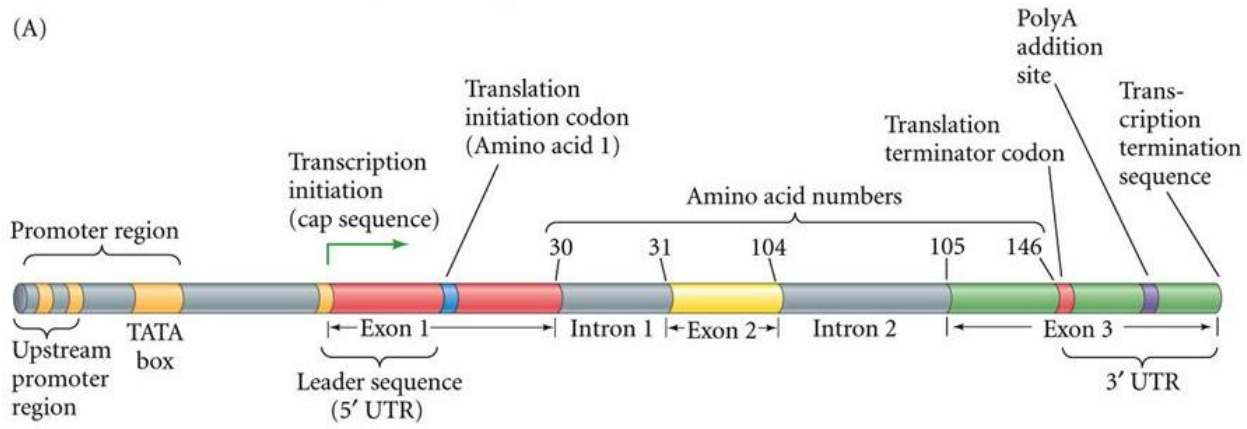
In the last lecture, we discussed how transcription proceeds in prokaryotes, now we will talk about transcription in Eukaryotes.

First, let's study the anatomy of a eukaryotic gene.



As we see in the previous figure, there is a promoter region. Downstream to the promoter region we have a transcription start site (which is a sequence where transcription initiates) and at the end of the gene we have a transcription termination sequence where transcription ends.

What is the sequence sandwiched between the transcription initiation sequence and the transcription termination sequence?

It consists of exons and introns, which are both transcribed; however, introns are spliced out and the remaining exons are joined together.

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RNA polymerases

What is the difference between RNA polymerases in Eukaryotes and Prokaryotes?

- Prokaryotes have only one type of RNA polymerase which has multiple roles such as functioning as a Helicase (Unwinding the DNA), adding nucleotides (elongation of RNA), as well as rewinding the DNA double helix left behind it.

- Eukaryotes have 3 different RNA Polymerases (I, II, III)
  a. RNA Polymerase I transcribes **rRNA** genes
  b. RNA Polymerase II transcribes protein-encoding genes (**mRNA**) and **microRNA**. (we will focus on this polymerase)
  c. RNA Polymerase III transcribes **tRNA** genes and only **one rRNA** gene.

-Eukaryotic transcription initiation must deal with the packing of DNA into nucleosomes. (This means that the structure of the nucleosomes/chromatin must be kept intact).

- Since Bacterial RNA polymerases have multiple functions, they don't need additional proteins to initiate transcription, while eukaryotic RNA Polymerases need a large set of proteins called general transcription factors (TF).

  They are called 'general' transcription factors because they assemble on all promoters used by RNA polymerase II (they work on all genes transcribed by RNA polymerase II), and these factors are named according to the RNA polymerase they are associated with (TFII with RNA polymerase II,TFIII with poly III….and so on).

NOTE: TFII has many subtypes (TFIIA, TFIIB…..). And each one has a certain purpose.

functions of the transcription factors:

1. Help position the RNA polymerase correctly at the promoter.
2. Aid in pulling apart the two strands of DNA to allow transcription to begin (Similar to the function of a helicase).
3. Push the RNA polymerase forward to begin transcription.

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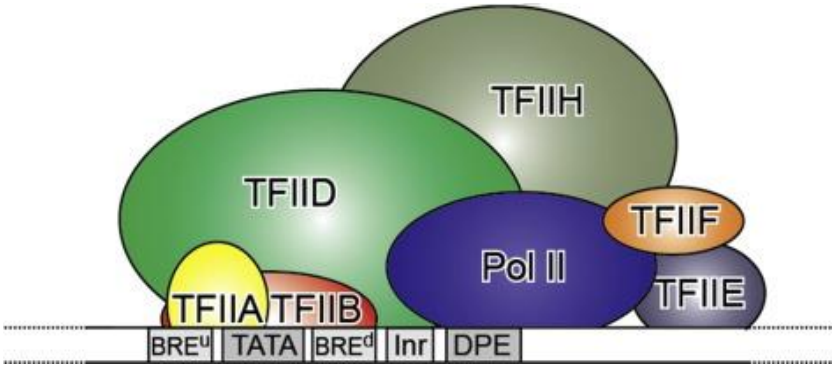
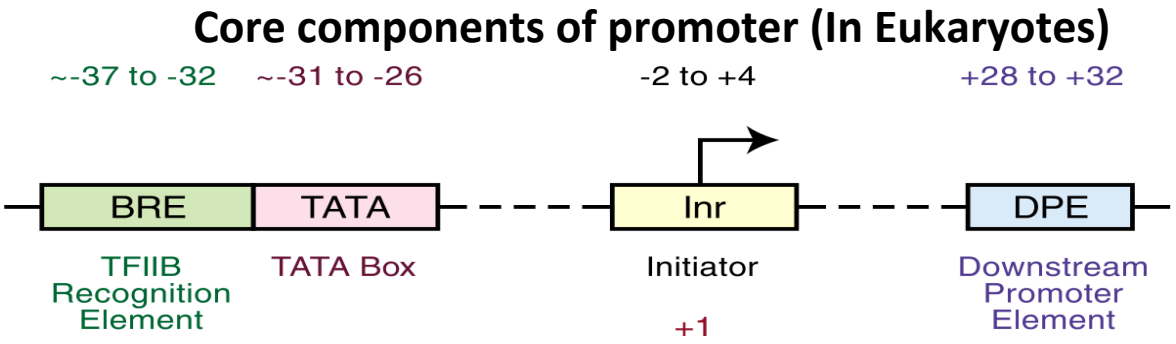
In order for transcription to begin, a preinitiation complex composed of RNA polymerase II and its associated transcription factors must be formed. This complex binds to the promoter.



The promoter region in eukaryotic cells is more complex than that of prokaryotic cells. How is that?



The promoter region is made up of different elements (consensus sequences) that are as follows:

1. BRE: TFIIB, which is part of the pre-initiation complex recognizes this element and binds to it.
2. TATA Box: It is similar to (-10) element in prokaryotes.
3. Inr (initiator): It contains +1 site where transcription initiates.
4. DPE (Downstream Promoter Element): it is part of the promoter and it is within the transcribed region.

*RNA polymerase is a large protein so when it sits on the DNA it can cover a large sequence including the DPE which is within the transcribed region, but the first nucleotide added is at (Inr).

Notes:

1. When we say factor, we are referring to a protein. On the other hand, when we say element, we mean a DNA sequence (a gene is an element)
2. The gene doesn't necessarily contain all four components, it may consist of any combination of these four (1,2,3,4), it could be one or two or four of them depending on the gene.

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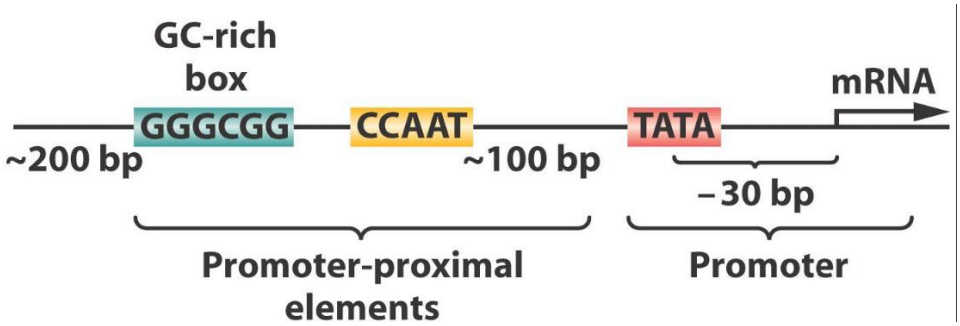
Promoter-Proximal elements (PPE)

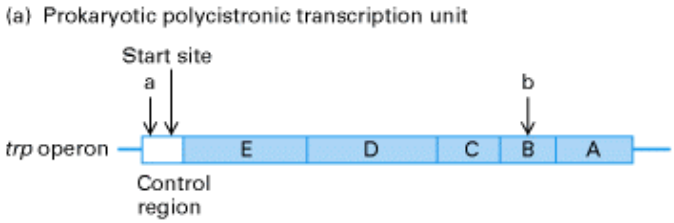


These elements (sequences) are upstream of the core promoter region, and they are important for strong expression (versus basal, and by basal we mean the 'default' or 'minimum' expression level of mRNA or Protein when we have preinitiation complex bound to the promoter).

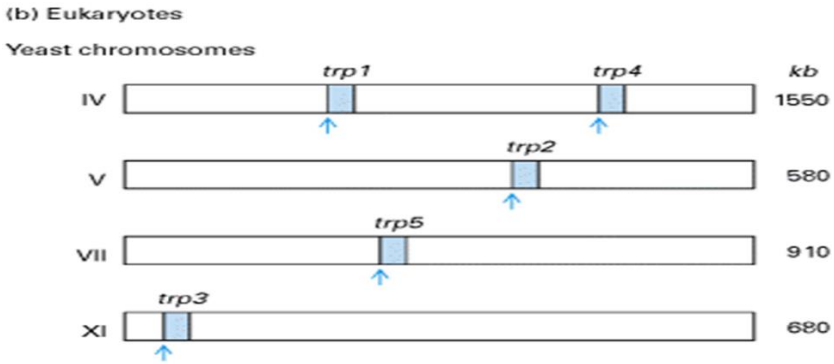
They are shared among different genes (gene-specific) that participate in a similar mechanism or needed for a particular purpose (example: production of enzymes for metabolism of glucose).

Note: PPE's are not found in all genes, they are found among a group of genes that participate in similar function or mechanism. Some genes only need the preinitiation complex to bind to the promoter for transcription to be initiated.

Operon vs Proximal-promoter elements



1. Operon: it is genetic material that gets transcribed into one mRNA that produces different polypeptides and eventually proteins (from different regions of mRNA) each with its own distinct function, but they all participate in the same mechanism.
   Ex: if the bacterial cell wants to synthesize tryptophan, it will synthesize a group of proteins altogether at once using the same operon (the same mRNA). Another example is the production of different enzymes that participate in metabolism of lactose.
   Note: operons are **prokaryotic *polycistronic*** transcription units.



2. Promoter-proximal elements: In eukaryotic cells, every enzyme is synthesized from a certain gene and these genes are located at different places (on different chromosomes or on the same chromosome but on different regions), each with its own core promoter. The related genes have the same promoter-proximal element adjacent (close) to the core promoter.

Ex (In the figure): Whenever the cell needs to synthesize and produce the enzymes necessary for the synthesis of tryptophan, they produce transcription factors (we are not talking about a single molecule, we are talking about thousands of the same factor). Once these factors bind to the PPE of the genes of these enzymes (trp1,2,3,4,5), all of these genes are transcribed **at the same time (simultaneously)**.

Pay Attention: At these genes, the preinitiation complex (including **RNA polymerase**) is bound to the promoter but RNA polymerase can't move unless specific transcription factors bind to PPE and activate the polymerase.

In bacteria, these enzymes are synthesized all at once from the same gene and the same mRNA(operon). Meanwhile, in eukaryotic cells each enzyme has its own gene, but they are controlled by the same PPE.

Tissue-specific transcription factors

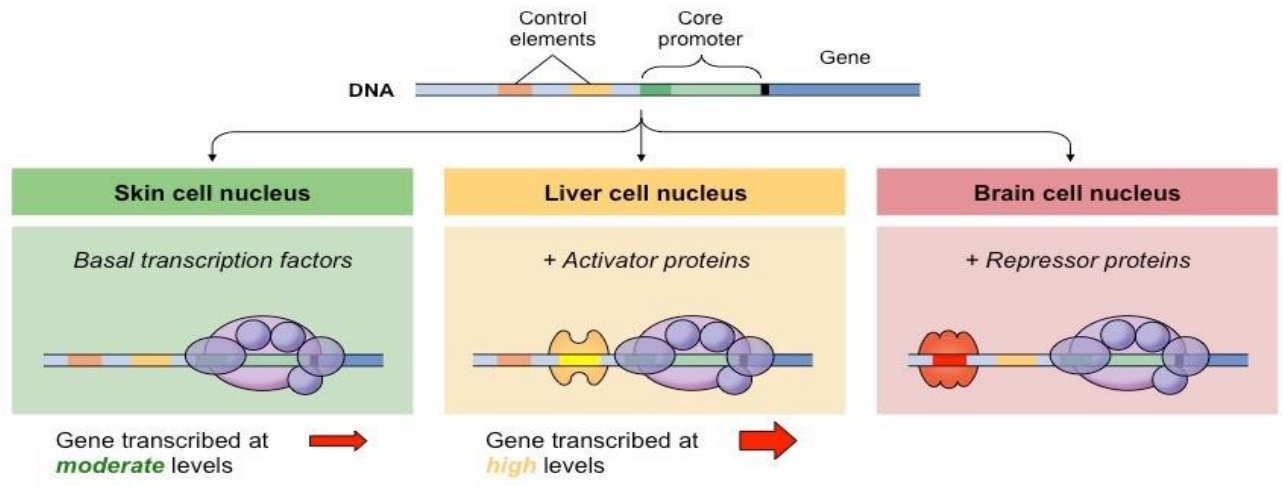
In our DNA, we have approximately 21,000 genes present in all tissues. Does that mean that all tissues produce the same proteins? NO. Insulin, for example, is produced in pancreatic cells and not in brain cells although the Insulin gene is found in both with the same promoter and the same PPE, why is that?

This is because the Insulin gene in brain cells is inactive and not expressed (transcribed) due to the absence of the needed transcription factors that **activate** transcription of insulin.

NOTE: General transcription factors are present in all cells, but each type of cell (or tissue) has its own **specific** transcription factors.

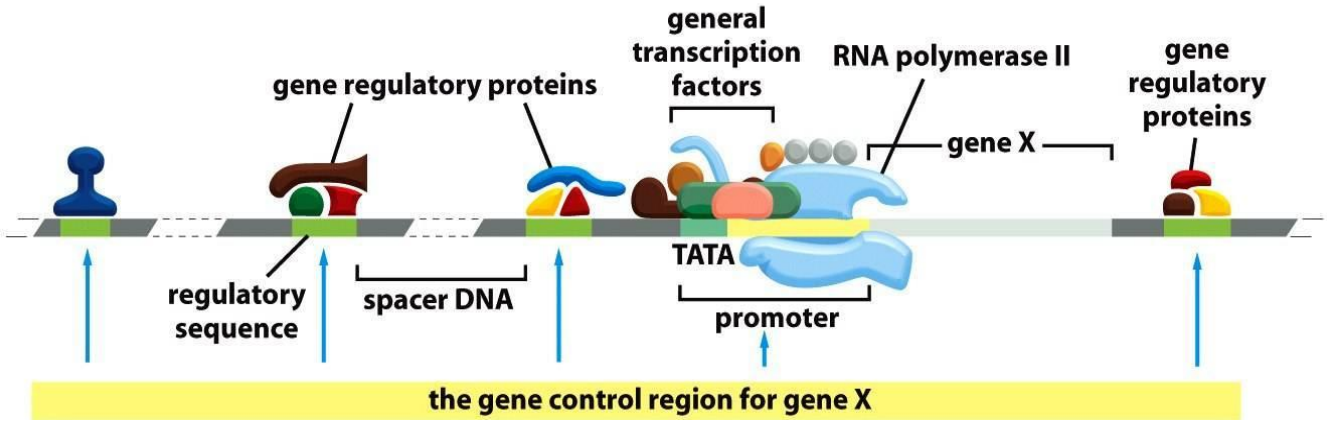
***Differential expression of transcription factors (tissue-specific transcription factors) determine gene expression.**

Consequently, different tissues could have different types of specific transcription factors for the same gene. These transcription factors can regulate the level of transcription for that specific gene. For example, liver cells have an activator (which is a TF) for a certain gene (it will be transcribed in high levels), while brain cells have a repressor (TF) for that same gene (no transcription happens). Skin cells don't have neither activators nor repressors for the gene, and will therefore transcribe it moderately (Basal level of transcription).

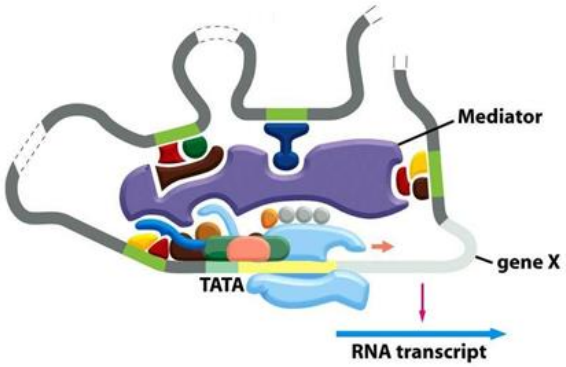


Activators and repressors bind to enhancers and silencers, respectively, so what are enhancers and silencers?

Enhancers



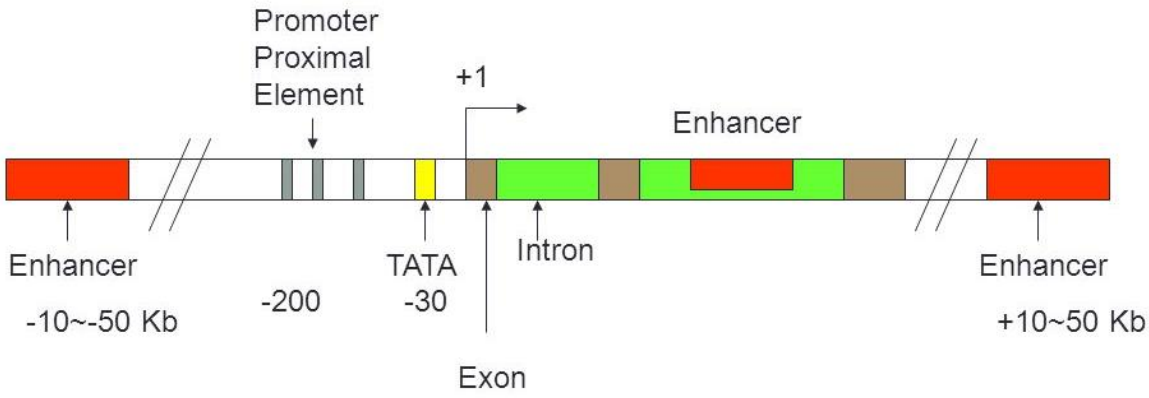
Many genes are regulated by cis-acting regulatory **sequences** called (enhancers), which are binding sites for **gene-specific** transcription factors that regulate RNA polymerase II such as a protein called the *Mediator*.



- These enhancers could be close or far away from the gene, can be upstream or downstream to it or in the gene itself, it also can be flipped and still be functional, so how can it regulate RNA polymerase II if it is far or flipped?
  -This is because DNA is a dynamic molecule and can loop around (DNA Looping) and this would help bring the enhancers close to the gene.

- When the DNA looping happens, the enhancer's proteins (activators) will bind to the promoter region proteins (RNA polymerase II and TF's). This binding is mediated by a protein called *Mediator*.

These enhancers push the RNA Polymerase to move forward (activating or stimulating transcription).



This figure shows that the enhancers could be downstream or upstream of the gene, or it can be inside the gene itself.

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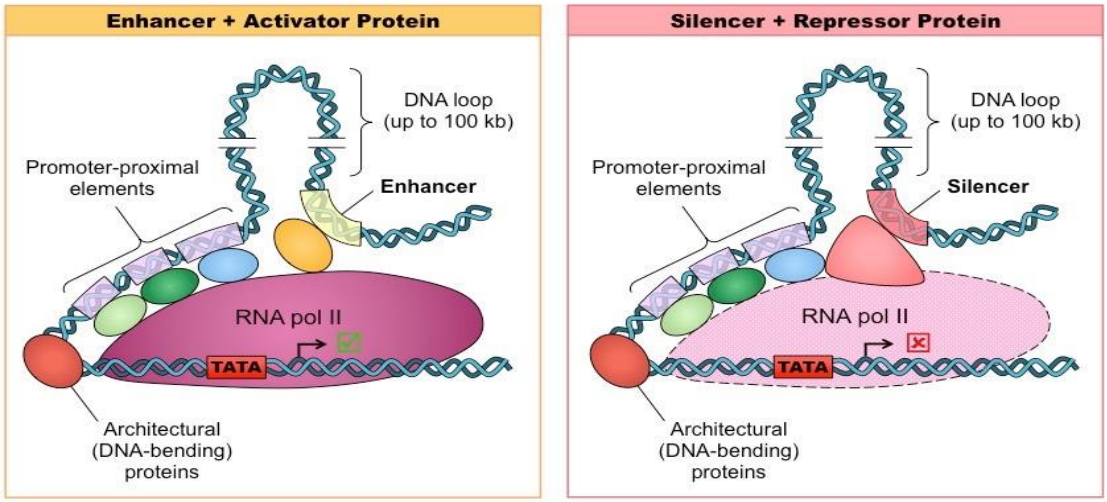
Silencers

Transcription in eukaryotes can be also regulated by sequences called *Silencers.* They operate by DNA looping, but have the opposite function of enhancers. They repress transcription by the binding of their proteins (repressors) to RNA Polymerase II and stopping its movement.

NOTE: Both the enhancer and the silencer are regulators of transcription.



Note:

Each gene has its own promoter region and it binds to the preinitiation complex, but it isn't necessary for the PPE's, enhancers, and silencers to be present in all genes.

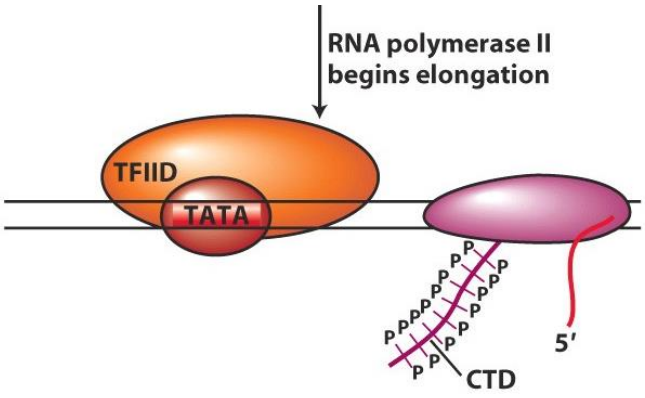
MECHANISM OF TRANSCRIPTION in Eukaryotes

Transcription proceeds in 3 stages:

1. INITIATION:

- The general transcription factor 'TFIID' which is a part of the preinitiation complex, binds to the promoter, recruiting other proteins (such as RNA polymerase and other transcription factors) to form the transcription preinitiation complex that we mentioned before.
- TFIIH, which is part of that complex, contains a DNA helicase and creates an open promoter exposing the DNA template to the RNA Polymerase.
- REMEMBER: (In bacteria, RNA polymerase functions as a helicase).

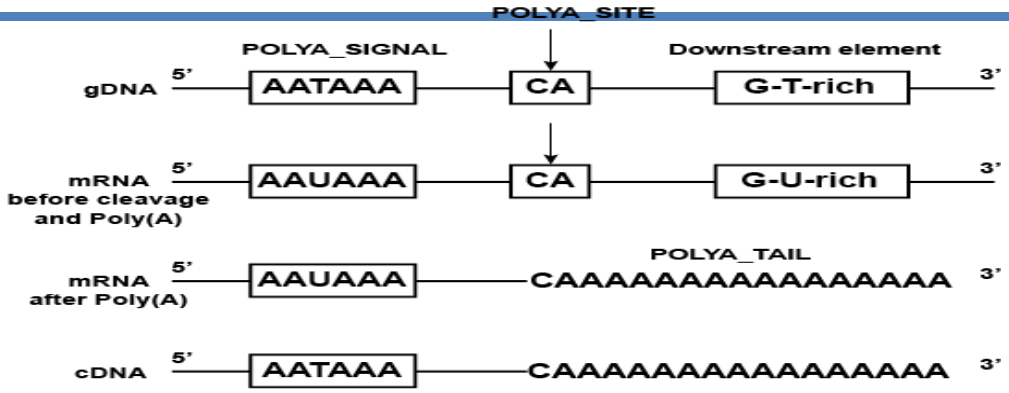
2. Elongation:



- RNA polymerase elongates the RNA by adding nucleotides, but first it needs to be phosphorylated.

- How is RNA polymerase II phosphorylated? Phosphate groups are added to the tail of it. TFIIH, which contains a protein kinase subunit, catalyzes phosphorylation.

Until now, we have mentioned three functions of TFIIH: it participates in the transcription coupled repair mechanism, functions as a helicase (initiation) and kinase (elongation).



3. Termination: Termination is determined by a consensus sequence for termination, which is AAUAAA followed 10-30 nucleotides downstream by a GU-rich sequence.

WHAT happens when RNA polymerase transcribes the last part of DNA into GU-rich sequence?

- RNA Polymerase stops and cuts the RNA between the CA, putting an end to the interaction between the DNA, RNA and RNA polymerase leading to the dissociation of the whole complex. The RNA molecule is then modified by the addition of approximately 200 A's (polyA tail) which is not found in the DNA. (The cleavage and addition of polyA tail is done on the 3' end)

Note: The cutting is done by different proteins, not the RNA polymerase. Also, the process of addition of polyA-tail is called **polyadenylation**.

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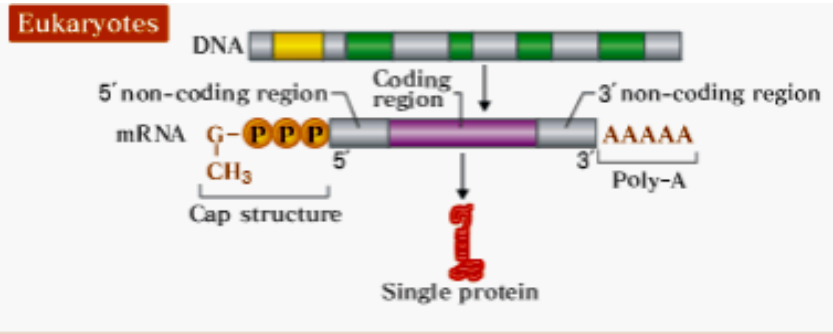
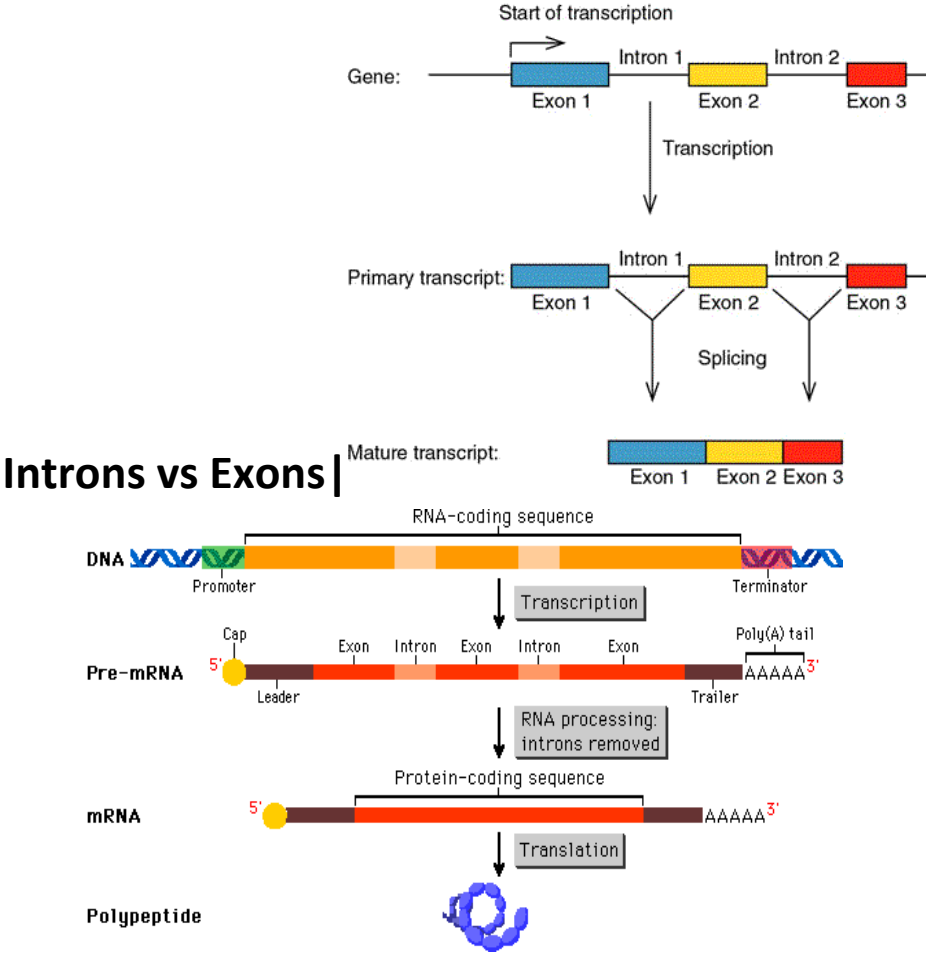
Eukaryotic Genes



Prokaryotic genes could be monocistronic or polycistronic (polycistronic like operons), but in eukaryotes genes are *monocistronic.*

That means that when these genes are transcribed and translated, every gene would produce only one polypeptide. (notice that it is polypeptide because it is not necessary to become functional protein).

Introns vs Exons|



Since introns are transcribed then removed they are not translated and are called “non-coding regions” (do not code for a protein)

Exons are called “protein-coding regions” because they are transcribed **and** translated.

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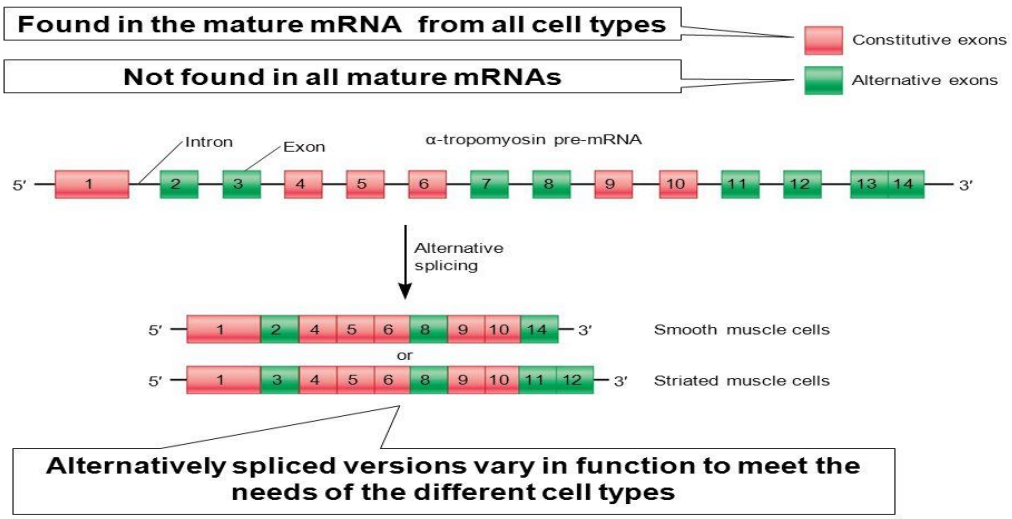
Alternative Splicing

Remember we said that we have only 21,000 genes in our DNA, but our cells have the ability to synthesize a much higher number of proteins. The reason for this is **Alternative Splicing.**

In this process, 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 but could differ in efficiency for example).



Pre-mRNA contains is made up of a number of introns and exons after it is formed.

In α-tropomyosin (in the previous figure), we have 14 exons and number of introns.

* If this protein is to be used in smooth muscle cells, introns are removed and exons 3,7,11,12,13 are also removed. The remaining exons are joined together to form a mature mRNA.

*If this protein is to be used in striated muscle cells, introns and exons 2,7,13,14 are removed.

So, cells use different isoforms of proteins in a different manner by splicing them by different ways.

Notice: if we have an exon that is 3’ to another exon, it can never be placed 5’ to it after splicing (the order of exons is maintained).

نعتذر عن أي أخطاء قد توجد في هذا الشيت ونرجو إخبارنا بها ومتابعة أي تعديلات تطرأ عليها، بالتوفيق جميعاً.