10

Molecular Biology

Doctor 2019 | Medicine | JU

Sheet Slides

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Contributed in the Scientific Correction

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Doctor

[redacted]

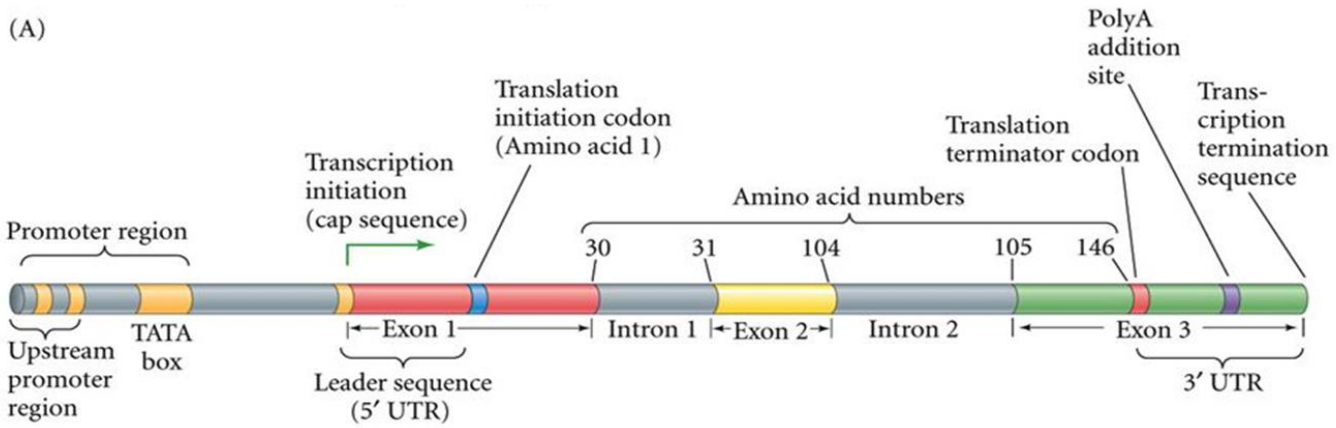
Dr. Walhan

Transcription in eukaryotes

Transcription in eukaryotes is much complicated than in prokaryotes. For example, bacteria RNA polymerase with the help of **σ** attach to the DNA and initiate the transcription. While in eukaryotes the RNA polymerase doesn't attach directly to the promoter, it needs the help of proteins called general transcription factors to bind to the promotor. So those proteins help the RNA polymerase to get a position in the DNA.

Another difference is that in eukaryotes there's exons (coding regions) and introns (noncoding regions with a regulatory functions) however they are both transcribed but introns will be removed later during SPLICING.

Anatomy of a eukaryotic gene



In the figure shown:

You can see that there's a eukaryotic gene with many DNA regions and sequences, to simplify if; there is a **promotor region** with different regions (upstream promotor region and TATA box) this region has an important role in initiation and regulation of transcription. **Transcription initiation site** where we find introns and exons. At the end of the gene, we have a **transcription termination sequence** where transcription ends.

We have both **5'UTR** untranslated region at the 5' end with a regulation role and **3'UTR** untranslated region at the 3' end with a regulation role.

RNA polymerases

In contrast to bacteria, which contain a single type of RNA polymerase, eukaryotic nuclei have three, called RNA polymerase I, RNA polymerase II, and RNA polymerase III.

What is the function of each RNA polymerase?

RNA polymerase I transcribes rRNA genes.

RNA polymerase II **transcribes protein-encoding genes (mRNA) and microRNA. We will focus on this.**

RNA polymerase III transcribes **tRNA** genes and one **rRNA** gene.

**mRNA is the code for proteins synthesis.

The function of tRNA and rRNA will be discussed furthermore in the translation lectures.

Remember that our DNA is packed into nucleosomes (negatively charged DNA wrapped around positively charged chromatin.

- Eukaryotic transcription initiation must deal with the packing of DNA into nucleosomes.

  Unpacking ⟶ **Transcription** ⟶ **Packing**.

- While bacterial RNA polymerase is able to initiate transcription *without* the help of additional proteins, eukaryotic RNA polymerases cannot.

  - They require help from general transcription factors. why do called them "general"?

  Ans: They are "general" because they assemble on all promoters used by **RNA polymerase II**.

  They are designated as **TFII** (for transcription factor for **polymerase II**), and listed as **TFIIA**, **TFIIB**, and so on.

General transcription factors

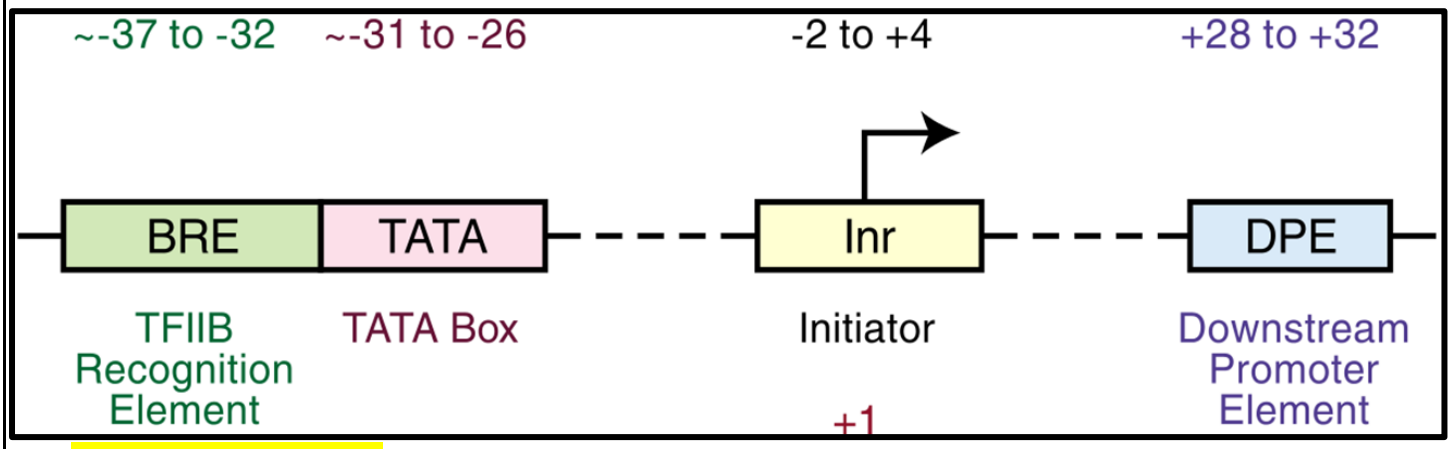
What is the function of these **general transcription factors**?

- help position the RNA polymerase correctly at the promoter. Similar to the function of σ in prokaryotes.
- aid in pulling apart the two strands of DNA to allow transcription to begin.
- push the RNA polymerase forward to begin transcription.

Remember: that in DNA replication the **PCNA** proteins the DNA polymerase to the primer region and DNA helicase has the function of unwinding the 3 DNA stands also.

Core components of promoters

The promoter region in eukaryotic cells is complex.



In the figure shown:

The promoter region in eukaryotic cells is more complex than that of prokaryotic cells. How is that? The promoter region contains different regions (consensus sequences) with different roles as follows:

1. **BRE**: TFIIB is the part of the pre-initiation complex that recognizes this element and binds to it.

2. **TATA Box**: recognized by TATA bindings proteins similar to (-10) element in prokaryotes.

3. **Inr (initiator)**

4. **DPE** (Downstream Promoter Element):

What do mean by (+), (-)

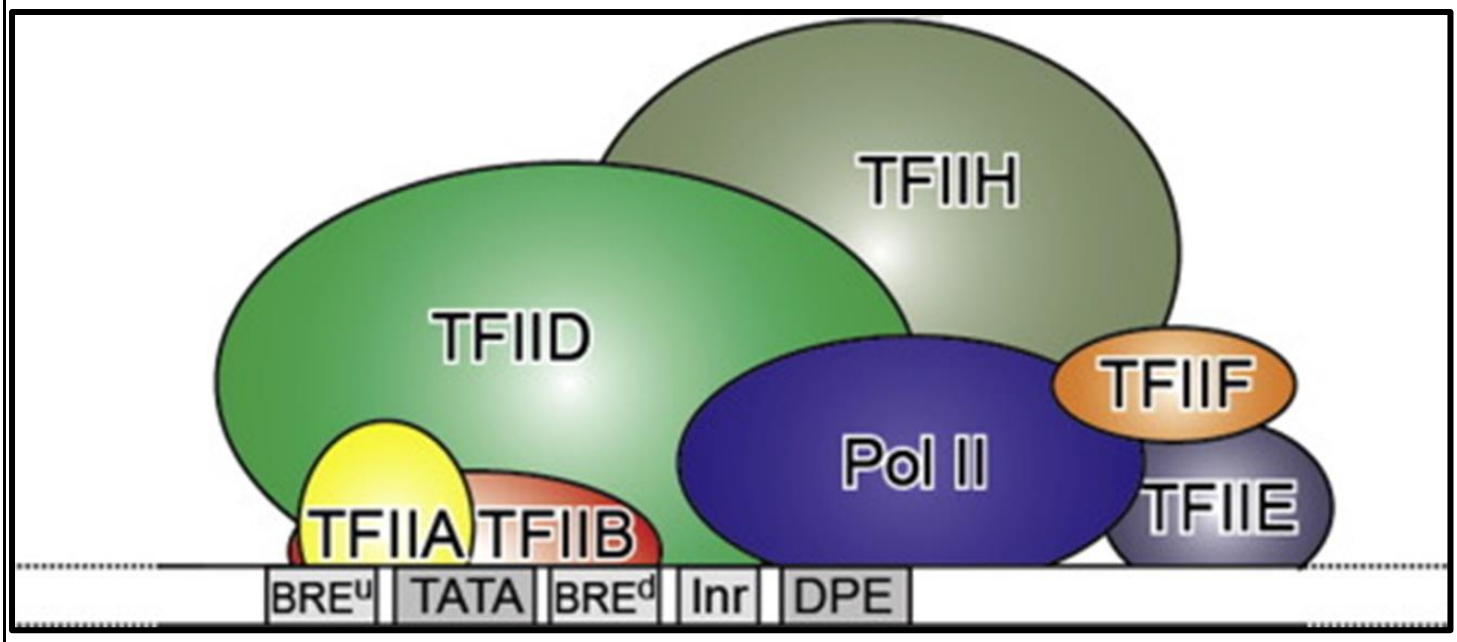
(+) means found downstream of the transcription start site.

(-) means found upstream of the transcription start site.

Note that the dr. Walhan said you have to memorize the numbers above.

**Not all of these sequences exist at once, but genes can have a combination of these promoter elements

Formation of preinitiation complex



**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.

In the figure shown:

We see the formation or the structure of the preinitiation complex containing: TFII A, B, D, E, F, and H

TFIIA: interact with the TATA binding protein

TATA binding proteins: is a subunit of TFIID and its responsible of recognizing and binding to the TATA box

TFIIB: recognize the BRE region in the promotor.

TFIID: bind to the TATA box through the TATA binding proteins and add some promotor selectivity.

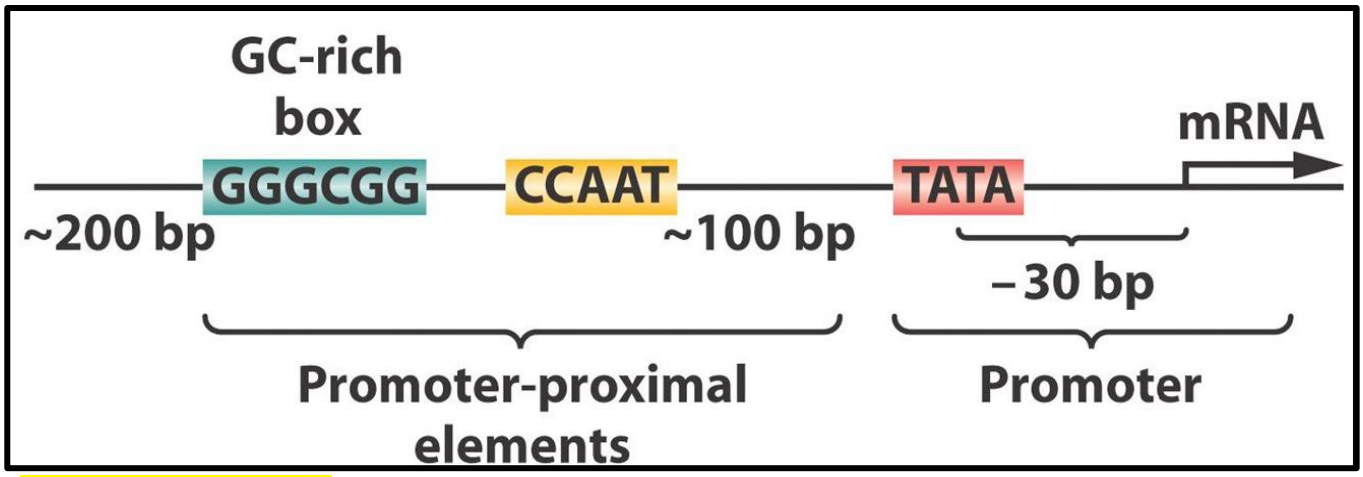
TFIIE: regulate and attract the TFIIH.

TFIIF: stabilizing the RNA polymerase interaction with the TATA binding proteins and TFIIB. Also help to attract TFIIE and TFIIH.

TFIIH: very important TF with a role unwinding the DNA double strand at the transcription start point. Have a kinase activity can phosphorylate the RNA polymerase at C-terminus to release the polymerase from the promotor region.

Note that: I asked Dr. Walhan if we have to memorize the function of each protein and each TF and he said we should.

Promoter-proximal elements



In the figure shown:

You can see PPEs a very important elements in regulating the transcription.

Examples on the PPEs is GGGCGG box and CCAAT box that are found upstream the promotor and the transcription start site.

These are upstream of the core promoter region. (200 bp upstream the transcription start site)

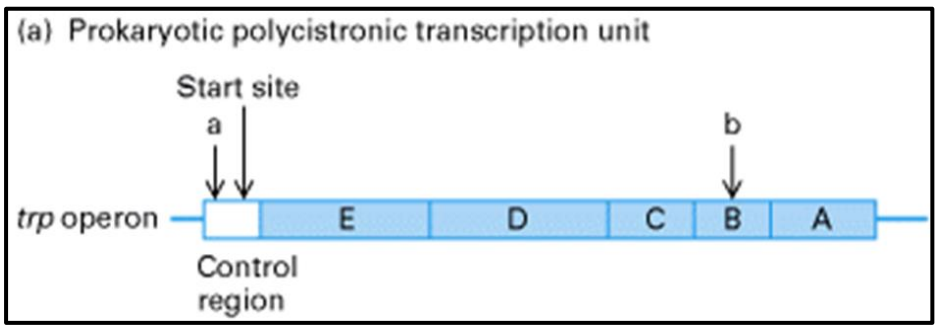
They are important for strong expression (versus basal).

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).

Alternative to operons!

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.

So what is the difference between the PPEs and operons?



Operon vs. Proximal-promoter elements

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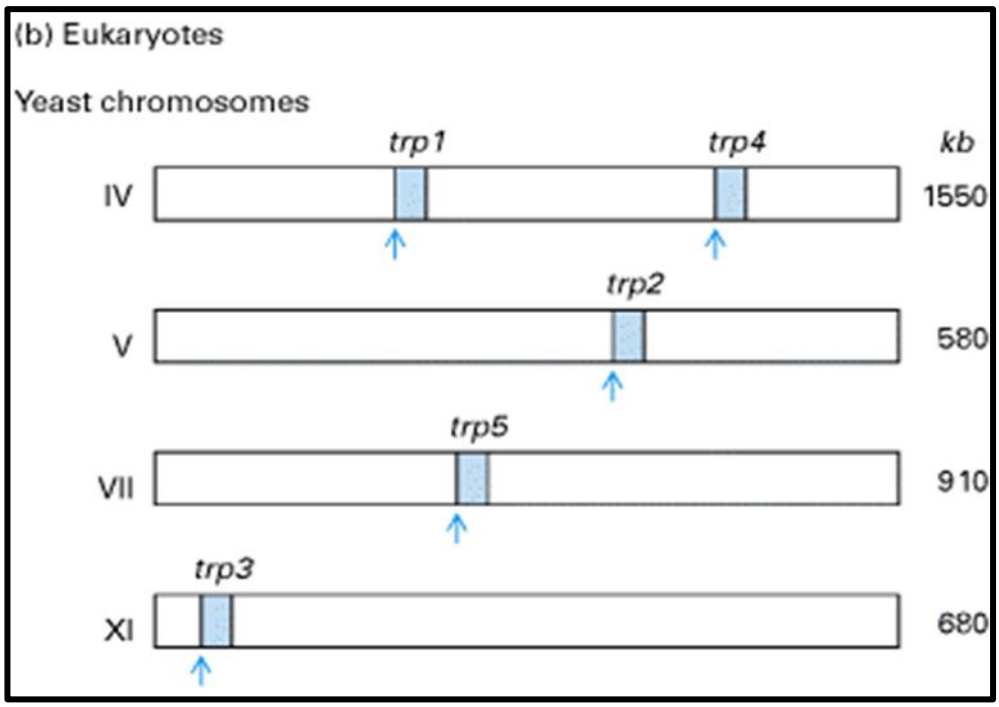
The operon is a functional unit of DNA containing a cluster of genes under the control of single promotor and usually involved in single mechanism



For both eukaryotes and prokaryotes we need ~~8~~ 5 enzymes to synthesis the **TRP.**

In prokaryotes all the 5 enzymes are expressed form a single transcription. (**polycistronic**)

In the figure shown:

In eukaryotic cells the 5 enzymes are expressed in an independent way in which one transcription unit for each enzyme. (**Monocistronic**)

See also the PPEs (proximal promotor elements) are shared among the DNA regulating the transcription.

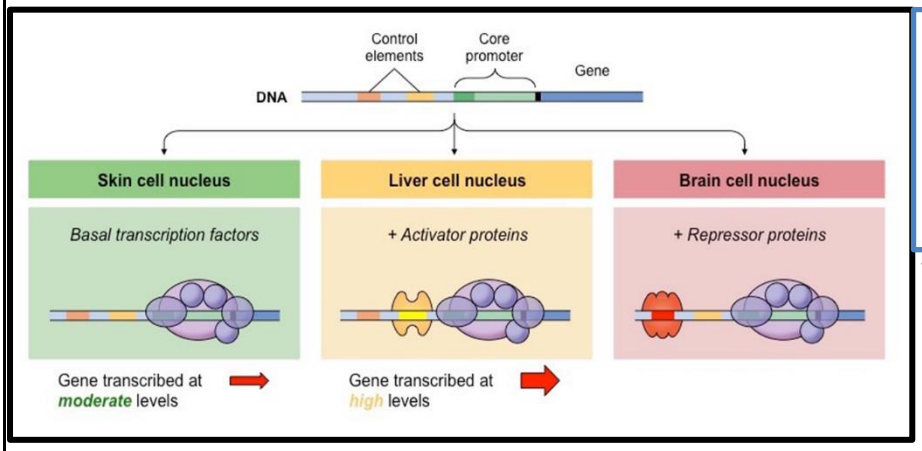
*** we have the same genes in all our cells so, are all genes expressed in the same way and at the same time and does that mean that all tissues produce the same proteins?**

NO. glucose , for example, is needed mostly in cells that need energy not all cells although the gene that mediate the energy production is found in all cells so how is

they are mediated to synthesis proteins related to energy production only in the cells that need energy the most .

This process is mediated by proteins called **Tissue-specific transcription factors**

Tissue-specific transcription factors



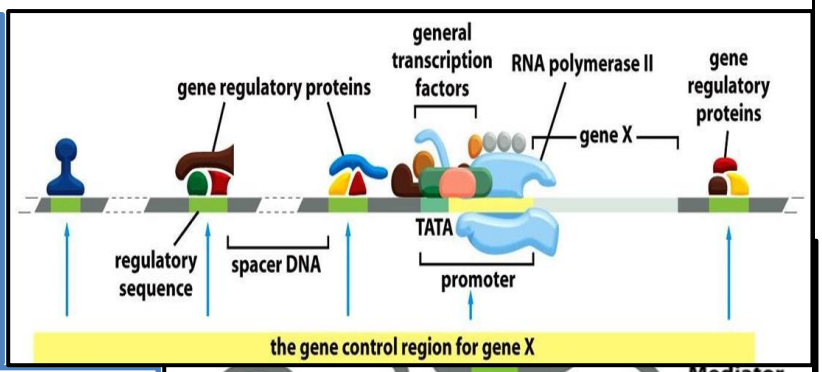
Differential expression of transcription factors (tissue-specific transcription factors) determine gene expression.

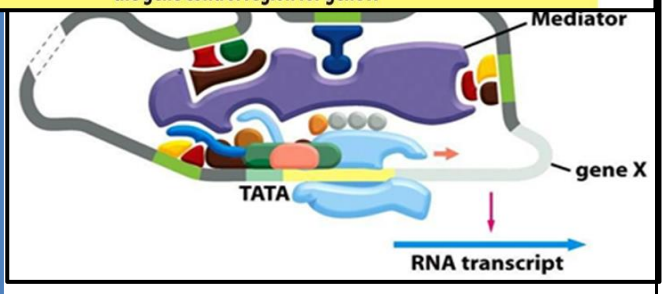
**Glucose metabolism occur in every cell in oor body except the Red blood cells because they are unnucleated

In the figure shown:
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. So for a certain gene involved in the transcription of insulin the skin cells the gene transcription is regulated at moderate levels by a Basal TF, in lever cells the gene transcription is regulated at high levels(where mostly needed) by proteins called Activators, in Brain cells (where insulin is not needed) no transcription happens because they have proteins called Repressors that inhibit the transcription.

Enhancers

Enhancers are short sequences of DNA they are recognized by proteins called Activators to increase the chances that a transcription of a certain gene will occur. Note: The Enhancers are located 10-50 kilo BP upstream the transcription start site.





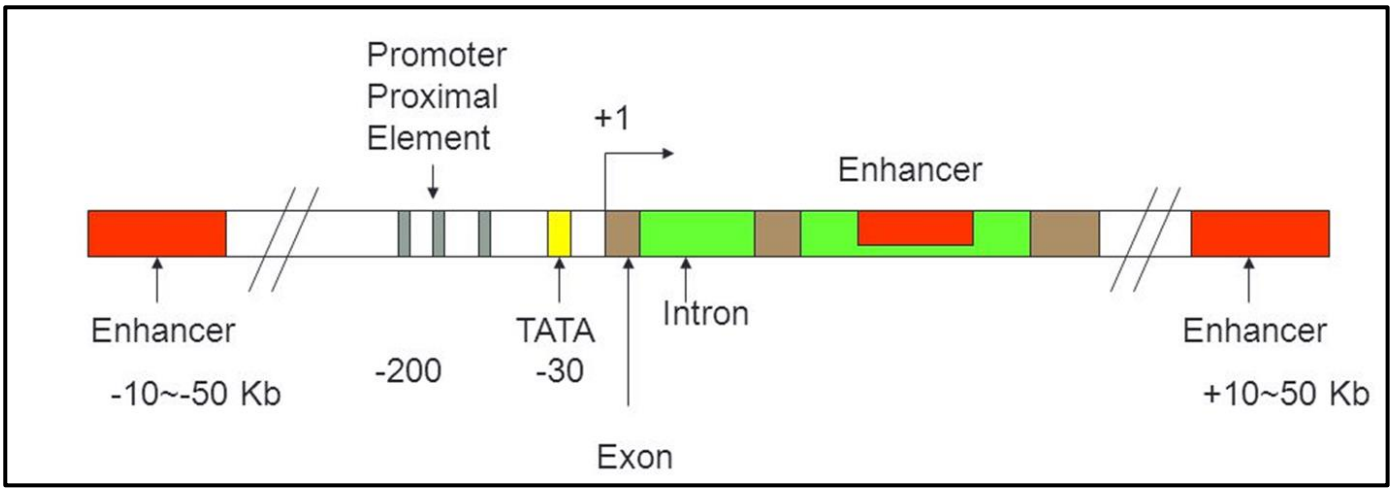
Many genes are regulated by regulatory sequences called enhancers, which are binding sites for specialized, gene-specific, cell-specific, regulatory transcription factors that regulate RNA polymerase II such as a protein called the Mediator.
They can regulate transcription regardless of orientation or location due to DNA looping

**** An enhancer may be located upstream or downstream of the gene it regulates**

- 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

In the figure shown:

A hypothetical regulatory sequences of a mammalian gene



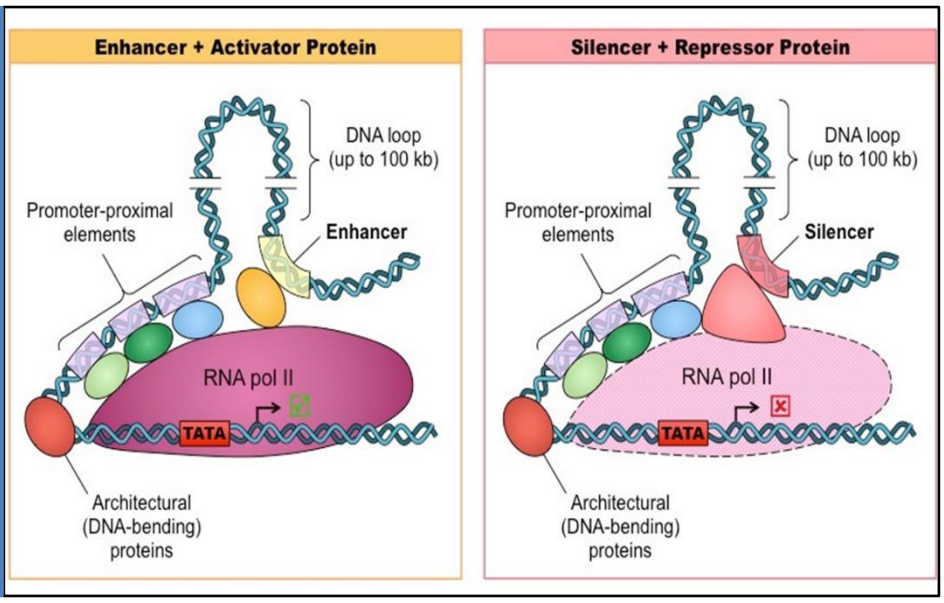
But what if we want the transcription to stop?

This process is mediated by proteins called **Silencers.**

Silencers

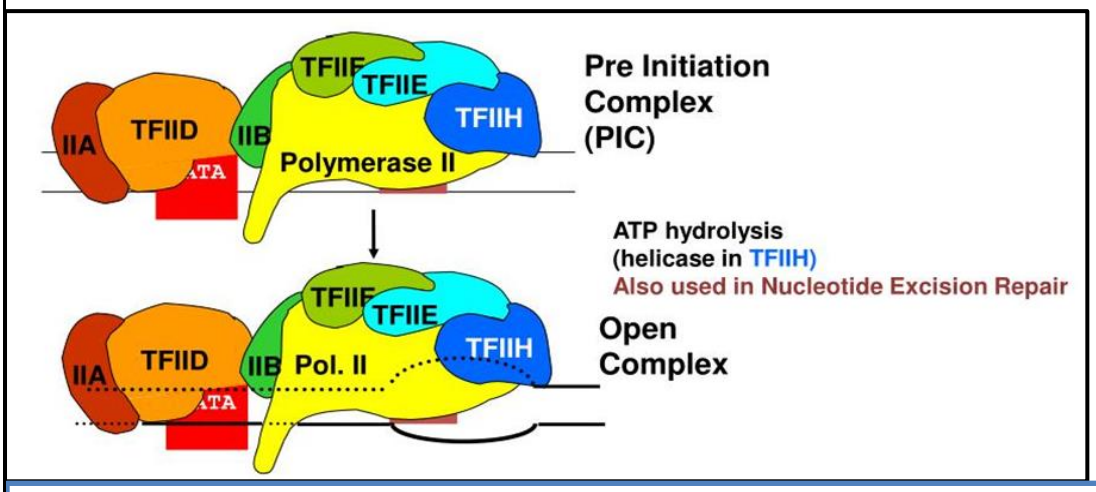
- **The opposite of enhancers**.

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



Mechanism of transcription

1-(initiation)



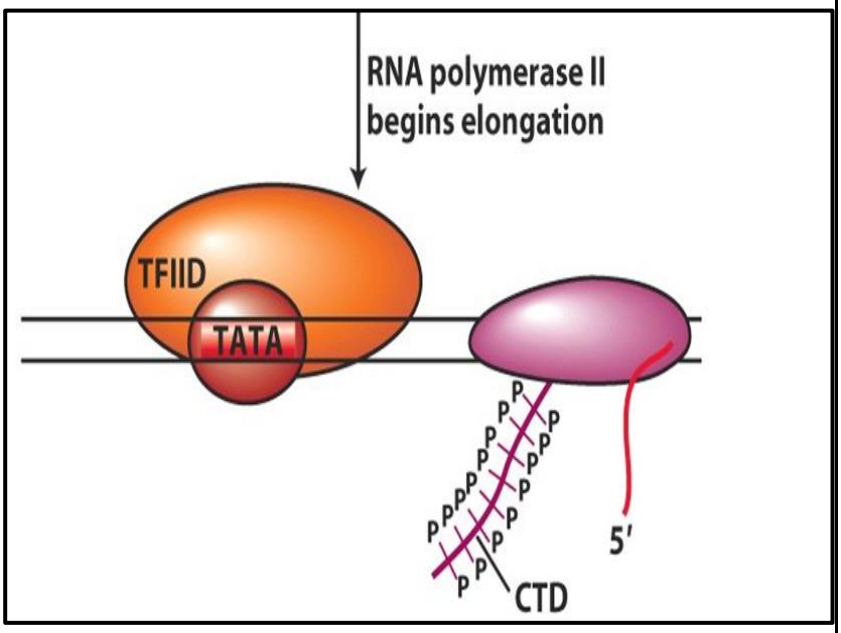
- TFIID binds to the promoter recruiting other proteins and forming the transcription pre-initiation complex.
- A member of this complex is TFIIH, which contains a DNA helicase.
    - TFIIH creates an open promoter exposing the DNA template to the RNA

**** note: TFIIH creates an open bubble exposing DNA to RNA polymerase to the synthesis if mRNA. Similar to the helicase activity in the DNA replication.**

2-(elongation)

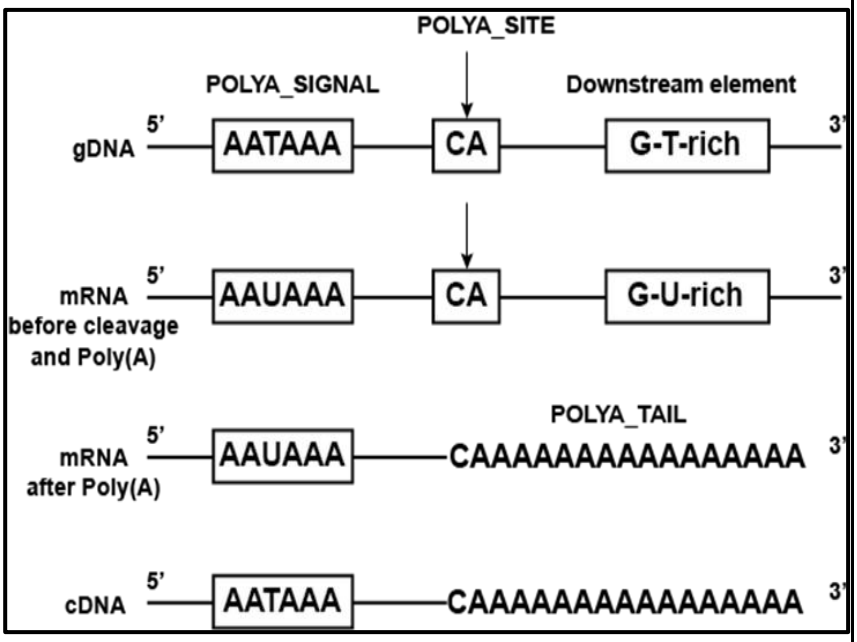
Movement of the polymerase is activated by the addition of phosphate groups to the "tail" of the RNA polymerase.

This phosphorylation is also catalyzed by TFIIH, which, also possesses a protein kinase subunit. Kinase: is an enzyme that catalyzes the transfer of phosphate groups from one molecule to another.

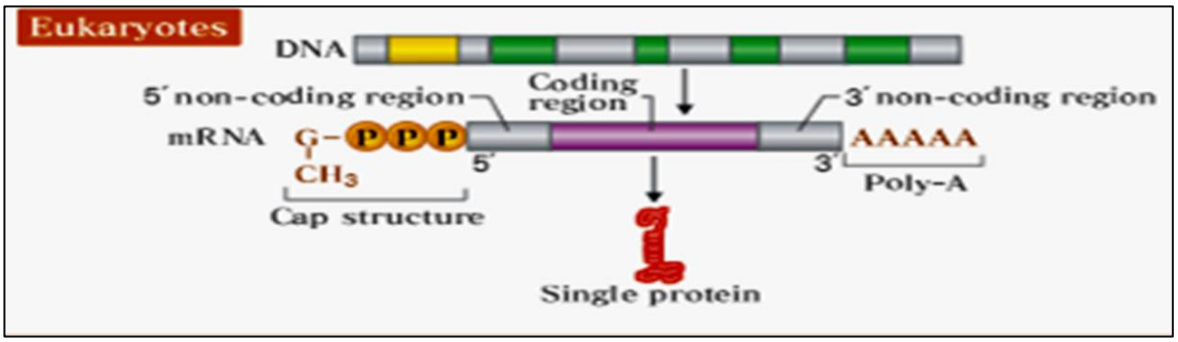


2-(termination)

Termination is determined by a consensus sequence for termination in mRNA, which is **AAUAAA** followed 10-30 nucleotides downstream by a **GU-rich** sequence .
What is the sequence in DNA? Try to write it with the correct directions.
Termination is coupled to the process that cleaves and polyadenylates the 3'-end of the transcript



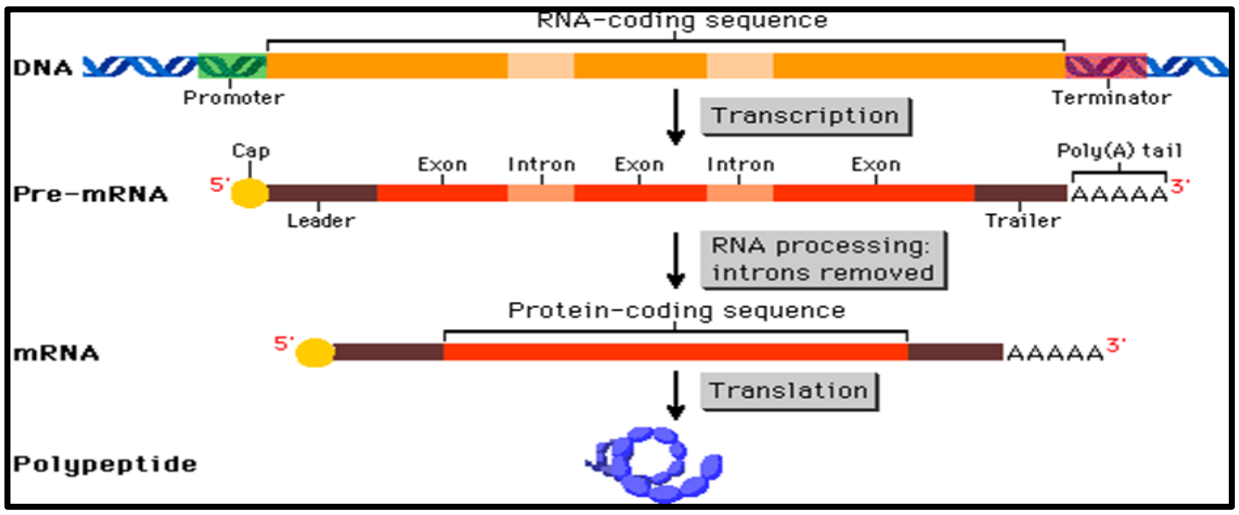
****Eukaryotic transcription units produce mRNAs that encode only one protein, thus termed monocistronic.**



Introns VS Exons

- The genomes of eukaryotic cells contain specific DNA sequences that do not code for proteins known as **introns**.
- The protein-coding regions are known as **exons**.
- When RNA is synthesized, the RNA molecule contains both **introns** and **exons** and is known as **pre-mRNA.**

** Later the pre-mRNA undergoes splicing which is removing the Introns and rejoining Exons to form a mature mRNA. Much more details about DNA splicing will be discussed in the next lecture.



Good luck

﴿لَا يُكَلِّفُ اللَّهُ نَفْسًا إِلَّا وُسْعَهَا ۚ لَهَا مَا كَسَبَتْ وَعَلَيْهَا مَا اكْتَسَبَتْ ۗ رَبَّنَا لَا تُؤَاخِذْنَا إِنْ نَسِينَا أَوْ أَخْطَأْنَا ۚ رَبَّنَا وَلَا تَحْمِلْ عَلَيْنَا إِصْرًا كَمَا حَمَلْتَهُ عَلَى الَّذِينَ مِنْ قَبْلِنَا ۚ رَبَّنَا وَلَا تُحَمِّلْنَا مَا لَا طَاقَةَ لَنَا بِهِ ۖ وَاعْفُ عَنَّا وَاغْفِرْ لَنَا وَارْحَمْنَا ۚ أَنْتَ مَوْلَانَا فَانْصُرْنَا عَلَى الْقَوْمِ الْكَافِرِينَ﴾ سورة البقرة- الآية 286