

FINAL – Lecture 10

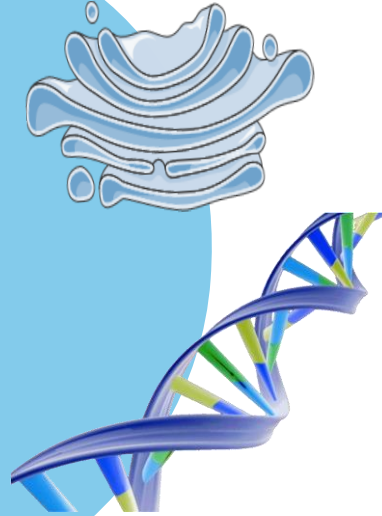
Transcription (Pt.3)

﴿ وَإِن تَتَوَلَّوْا يَسْتَبَدِلْ قَوْمًا غَيْرَكُمْ ثُمَّ لَا يَكُونُوا أَمْثَلَكُمْ ﴾

اللهم استعملنا ولا تستبدلنا

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Let's recall how we ended up here:

- God have created Adam and then Eve, they've committed the first human sin... Oh sorry! let's get to the current timeline.. Naively, you choose (or chosen to be) in this school (you've committed the sin of your life) so you basically trying to cope with it and a whole year has already passed and you are probably still trying to cope with it....Sorry!

Now seriously:

Transcription is the process of making RNA from DNA

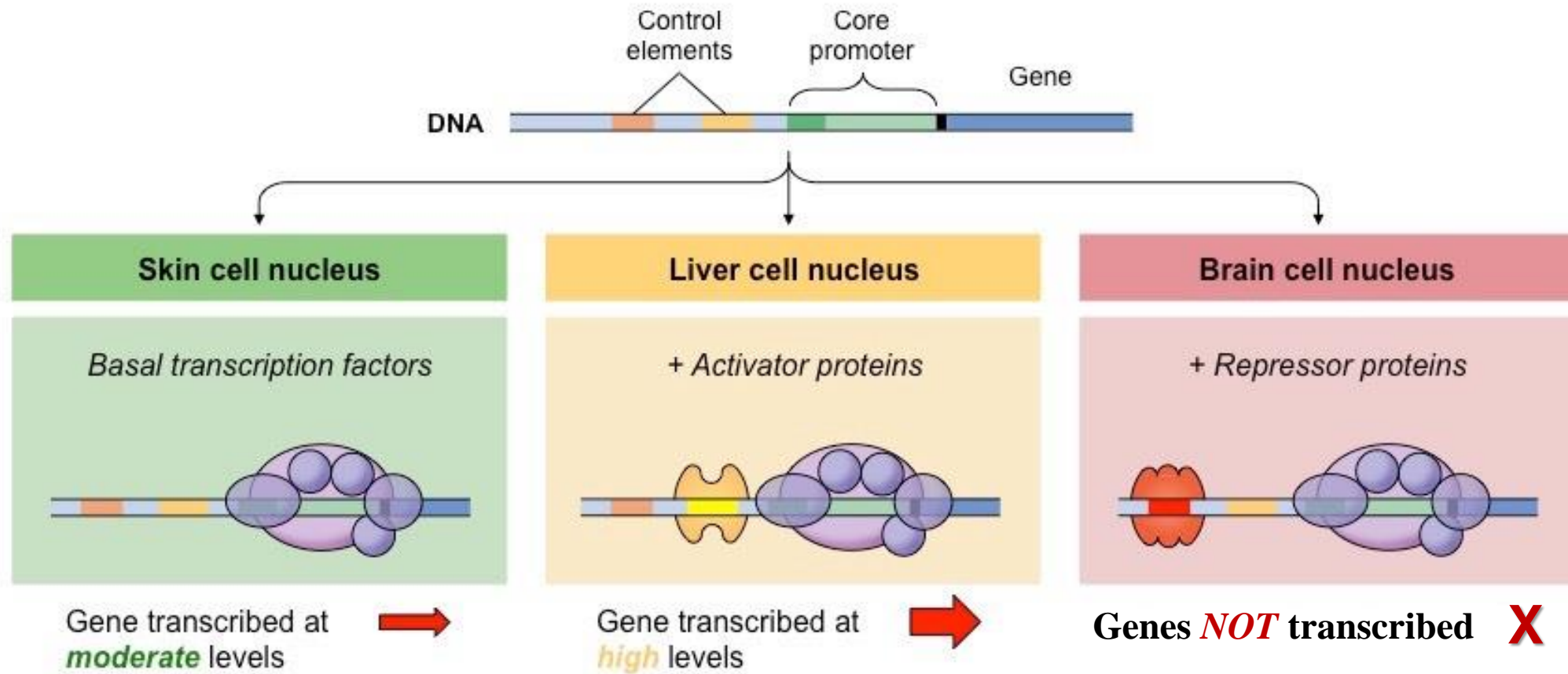
it follow a general mechanism:

1. Initiation: where RNA polymerase bind to a promoter sequence at the DNA
 - either by itself (in prokaryotes, and we've finished that)
 - by the help of transcription factors (in Eukaryotes, and we started the talk about Regulatory regions of DNA—like core promoters, proximal promoter elements (PPEs) -a lot of examples about it were given- enhancers and silencers). Also, we started talking about how transcription factors can be specific for certain tissue.
2. Elongation
3. Termination

In this lecture:

- completing transcription factor tissue specificity and regulatory regions of DNA (particularly: enhancers and silencers)
- chromatin immunoprecipitation technique
- Eukaryotic RNA processing (a process following transcription process)

Tissue-specific transcription factors



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

Specificity of transcription factor leads to specificity in transcription process, something determined at the moment of fertilization and start of cell division! And that's what makes tissue specific characteristics.

Another regulatory sequences in the DNA are:

Enhancers

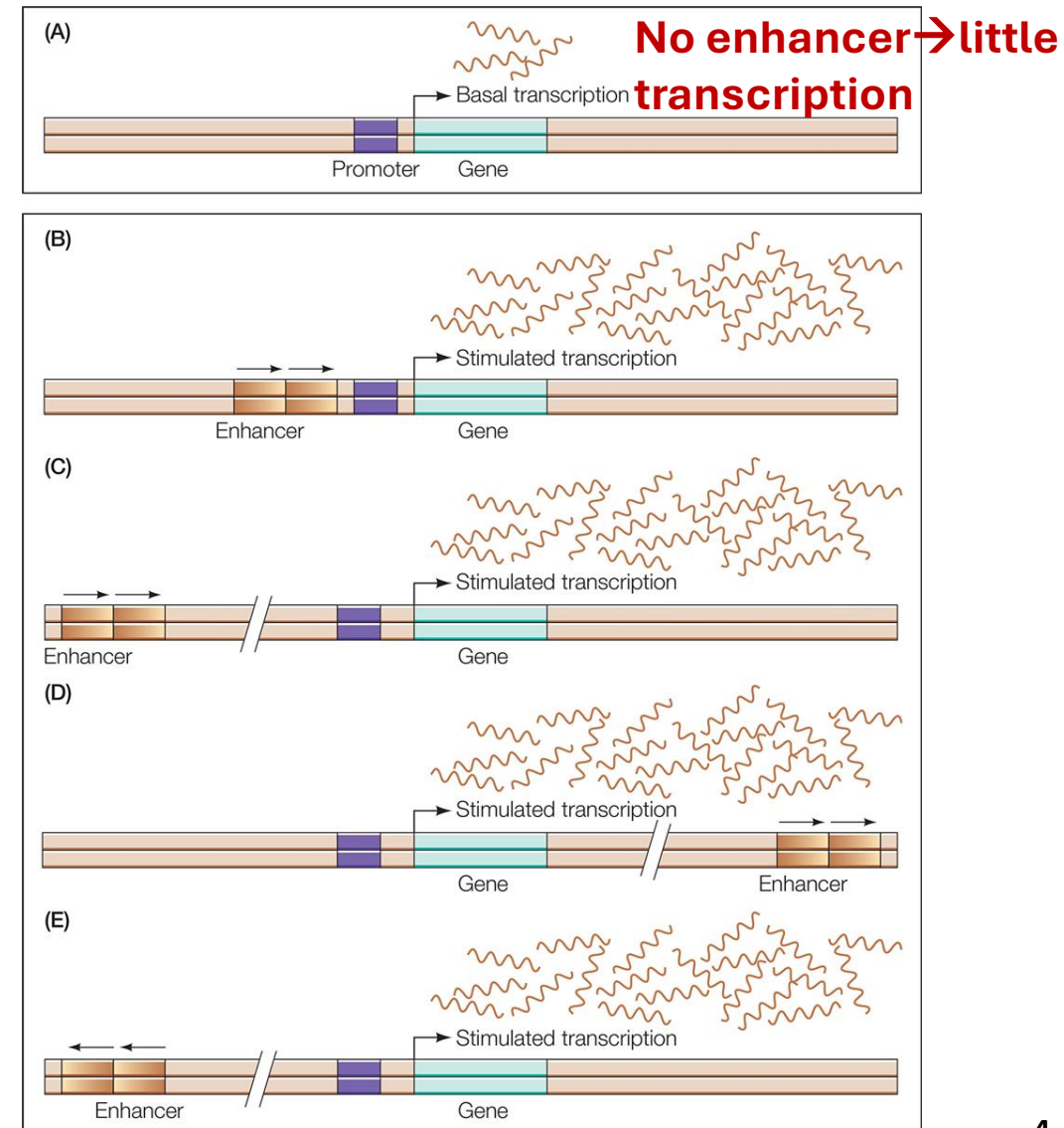
As the name implies enhancers control gene expression in a positive manner, that is: it enhance transcription.

- 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.
- They can regulate transcription regardless of orientation or location, **they can even be flipped and still be functional** due to DNA looping.
- There are 500,000 to over 1 million enhancers in the human genome, accounting for 10% or more of total genomic DNA.

The range 500k- 1M seems to be so wide!

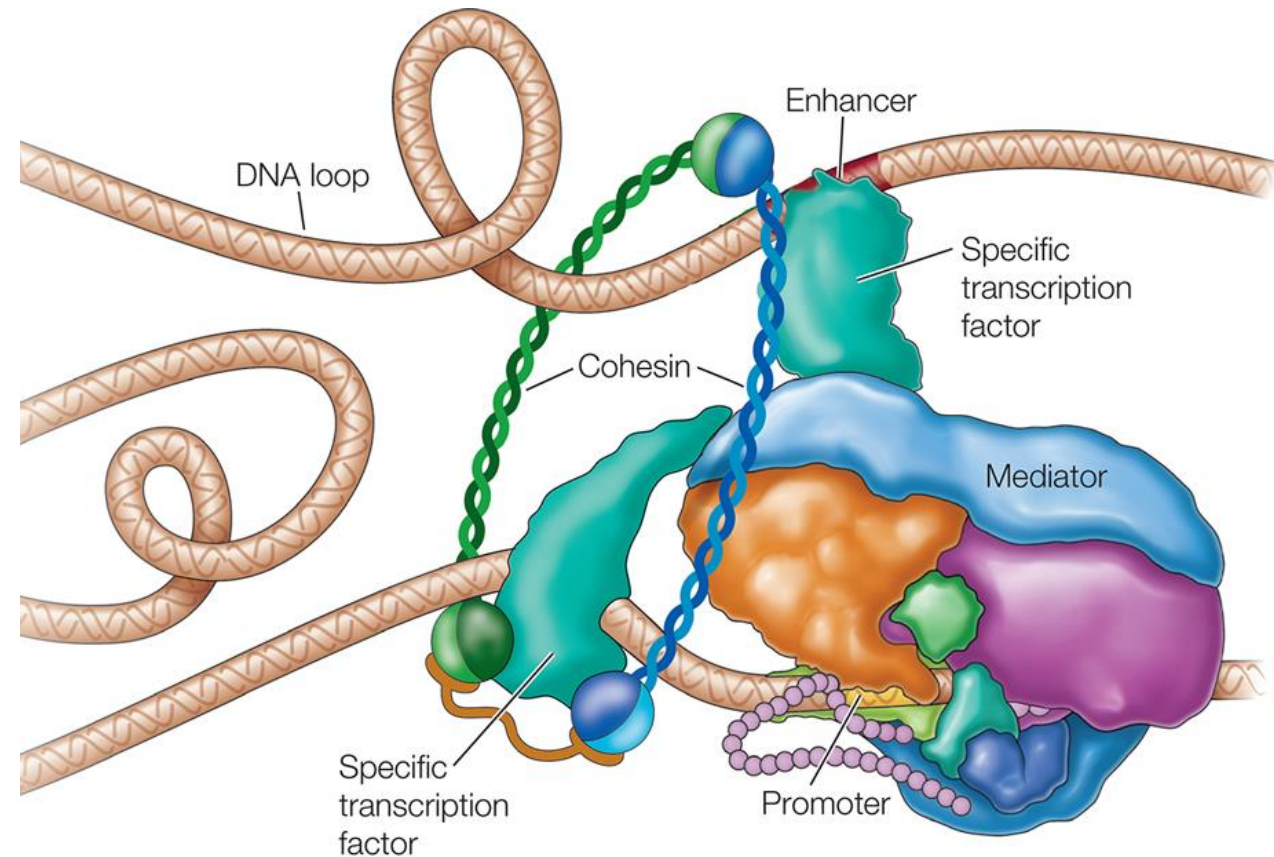
Simply because; they are not all accurately identified.

Basically, scientists look for patterns (i.e., consensus sequences) and notice the effect of these sequences on the process, so it becomes a probability matter, some sequences are confirmed 100% to be enhancers while others are doubted about.



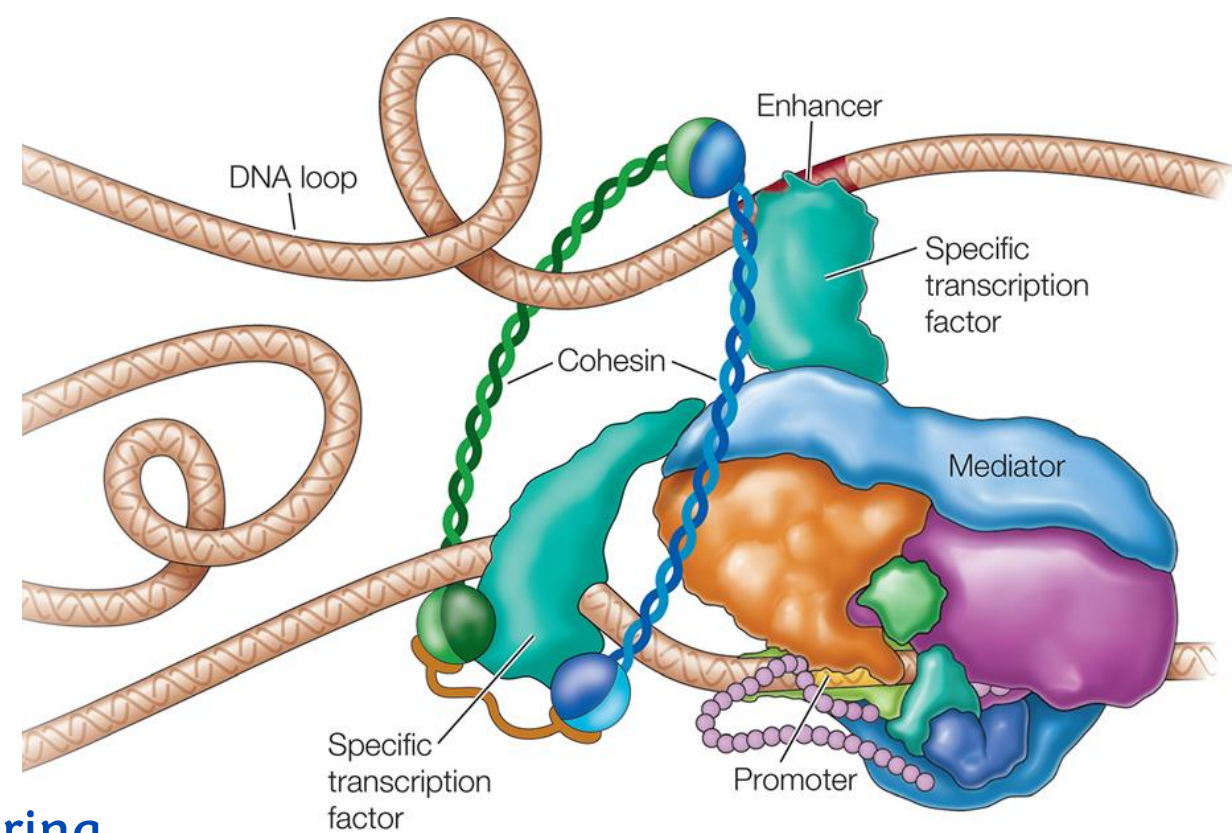
DNA looping and Cohesin

- Transcription factors bound at an enhancer can interact with a protein called (Mediator) or general transcription factors at the promoter.
- This is due to the ability of DNA to loop.
- DNA looping is stabilized by a protein called Cohesin.



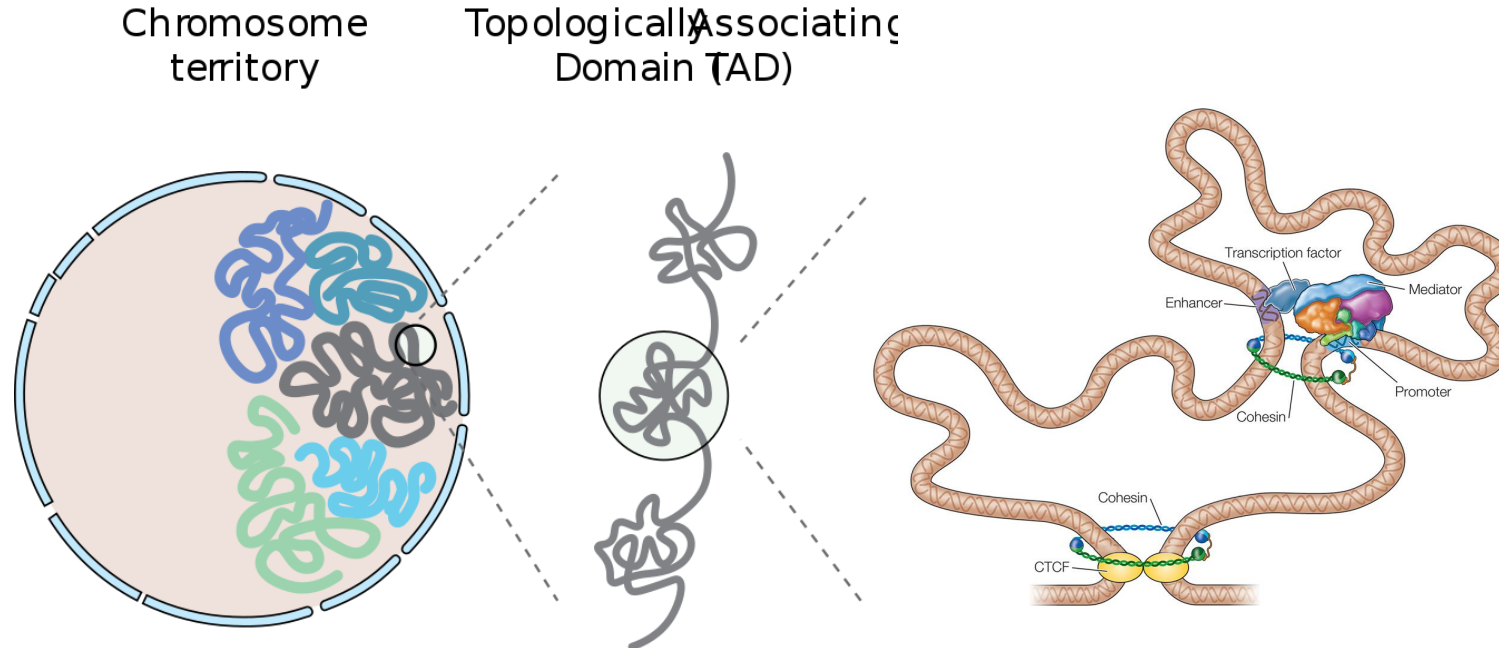
Regarding the previous slide

- Mediator protein interacts both with general transcription factors and with RNA polymerase and play role in linking the general transcription factors to the gene-specific transcription factors (which interact with enhancers) that regulate gene expression.
- The loops are stabilized by cohesin, which forms a ring structure that encircles two strands of DNA. Transcription factors bound to distant enhancers can thus work by the same mechanisms as those bound adjacent to promoters.
- Note that: Both DNA looping and mediation rely on protein-protein and protein-DNA interactions, which are non-covalent in nature. These interactions ensure precise communication between regulatory elements and the transcription machinery.



Enhancers, insulators, TADs, and CTCF

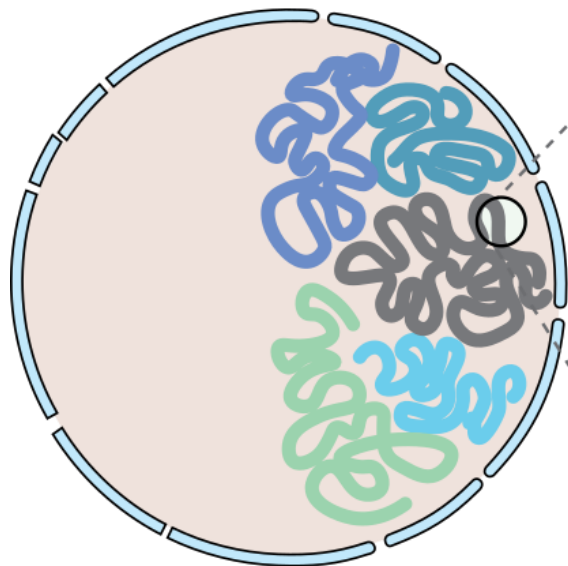
- So because DNA is dynamic Enhancer located on one end of chromosome can regulate a gene located at the other side?
 - Of course, **no** the process is regulated and restricted
- DNA sequences or elements known as insulators divide the genome into topologically associating domains (TADs) forming loops.
- The boundaries of the loops are stabilized by cohesin and CTCF proteins.
- Enhancers are restricted to interacting with promoters in the same domain.



- How is it restricted

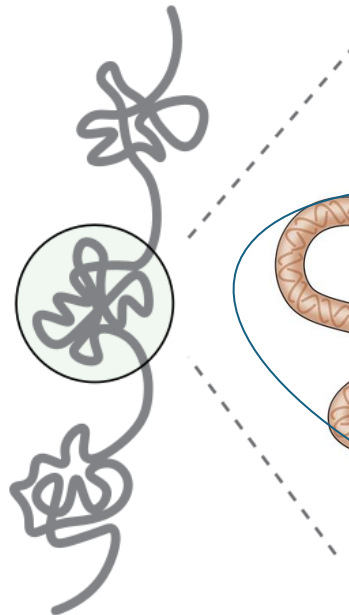
DNA is organized as heterochromatin and euchromatin regions

Chromosome territory

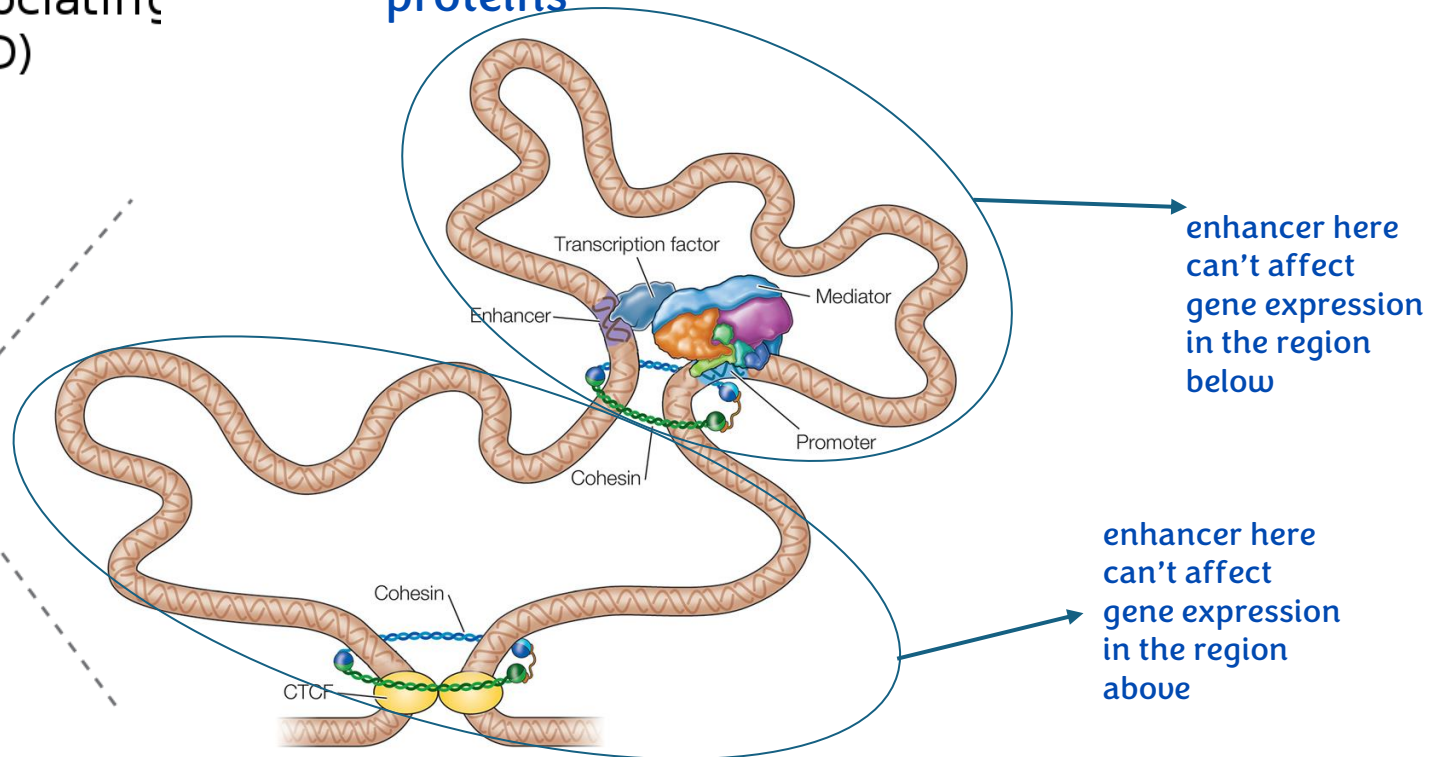


These regions are further organized into Topologically associating Domains

Topologically Associating Domain (TAD)

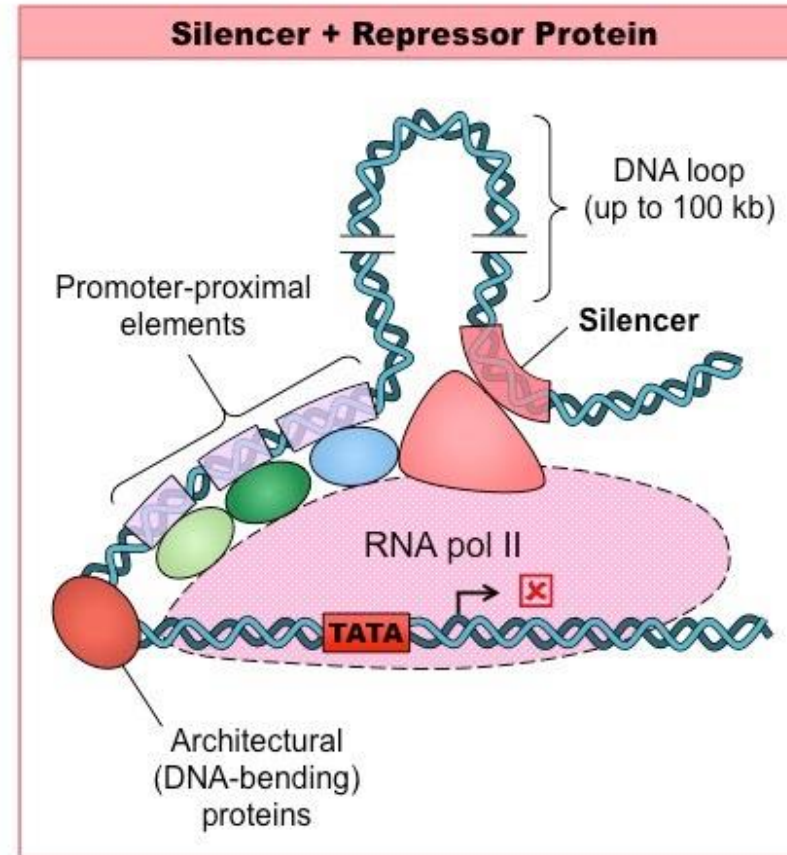
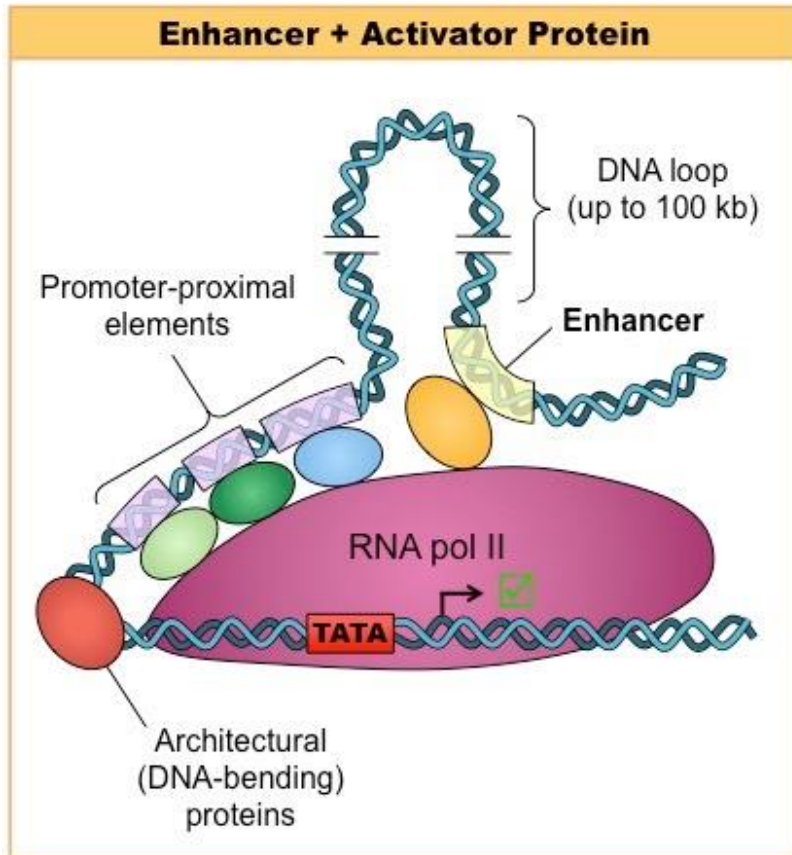


These regions are further divided into smaller regions separated by cohesin and CTCF proteins



Silencers

- The opposite of enhancers. While enhancers up regulate expression of gene, silencers suppresses it



Silencers are thought to have same characteristics as enhancers:

- They can regulate transcription regardless of orientation or location, they can even be flipped and still be functional due to DNA looping.
- they are binding sites for specialized, gene-specific, cell-specific, regulatory transcription factors that regulate RNA polymerase II

Regulatory Elements Summary Table

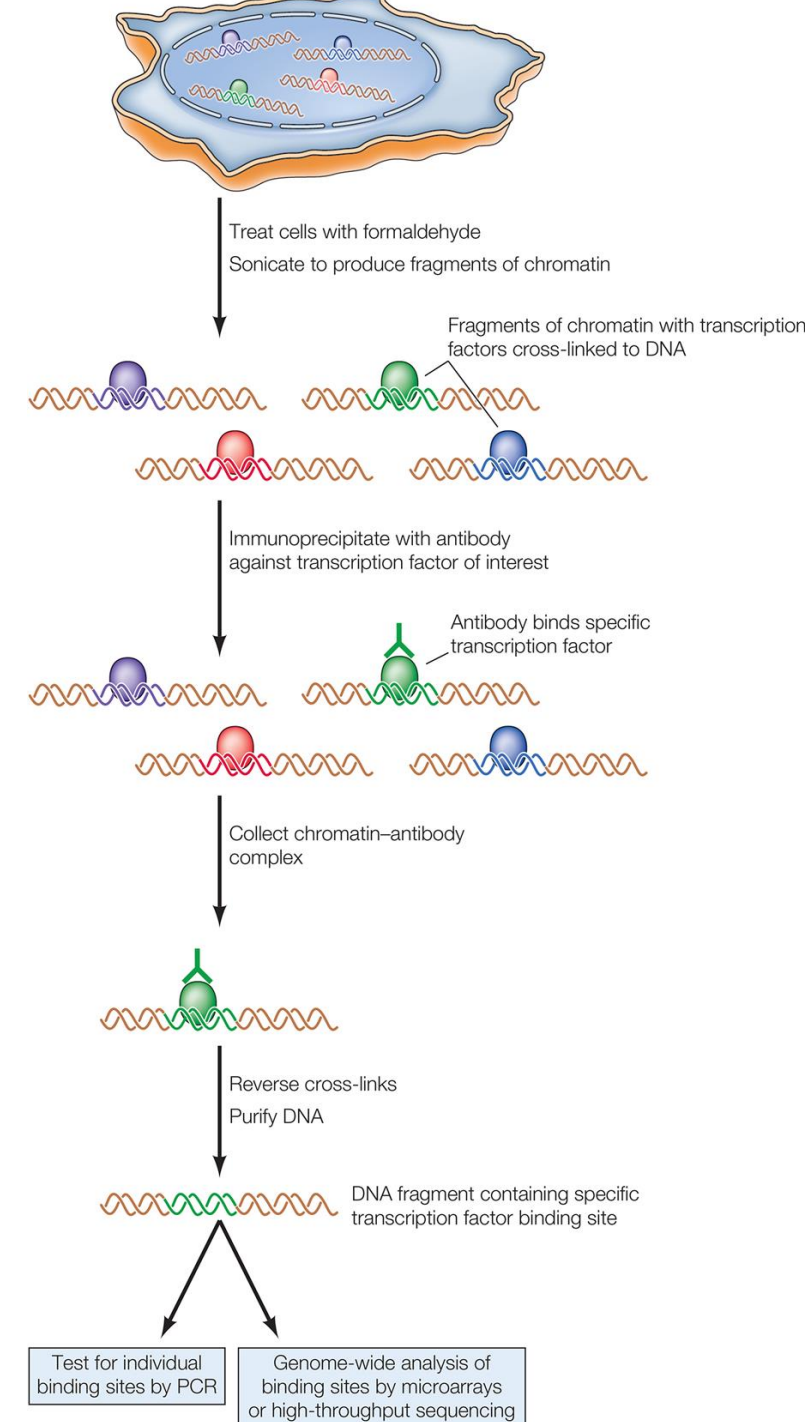
Regulatory Element	Location	Function	Effect on transcription activity
Enhancers	Can be far from the target gene (upstream, downstream, or within introns).	Bind activators to enhance transcription of a specific gene, even from a distance.	Enhancers amplify the activity initiated at the core promoter
Silencers	Similar to enhancers (upstream, downstream, or intronic).	Bind repressors to suppress transcription of a specific gene.	Silencers can inhibit the function of the core promoter
Promoter-Proximal Elements (PPEs)	Close to the core promoter, usually within 200 bp upstream of the TSS.	Bind specific transcription factors to regulate transcription near the start site.	help fine-tune transcription
Core Promoter Regions	Directly adjacent to the TSS (transcription start site).	Bind general transcription factors (GTFs) and RNA polymerase to initiate transcription.	provide basal (minimal) transcription machinery

- Please be aware that this schedule is for understanding purposes, some info aren't mentioned here or in previous lecture

How we identify the previous sequences? One technique is:

Chromatin immunoprecipitation

- 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.
- DNA is isolated and fragmented.
- 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.



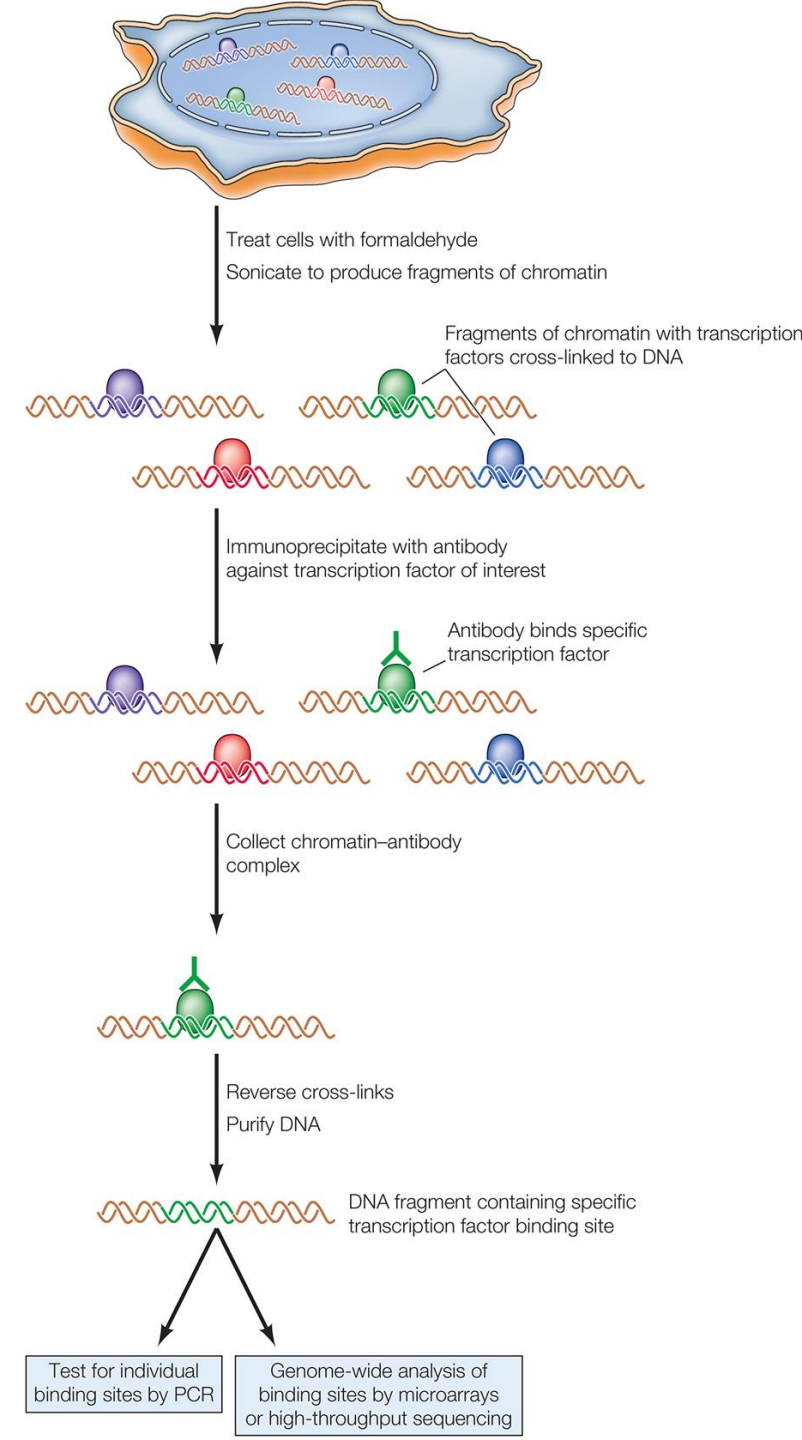
how antibodies form against these proteins?

- proteins are injected to another organism so, it will be identified as foreign material, which lead to production of antibodies specific to these proteins.

We are interested in knowing the specific sequence that the proteins bind to at DNA.

1. We take cells growing in vitro, and we cross-link proteins to DNA by certain chemicals (making a covalent bond, rather than the natural non-covalent bonding) so proteins are stuck to the DNA.
2. DNA is extracted from cells, then fragmented (random fragmentation, between proteins binding to DNA). Here no fragmentation occur to the protein bound sequence since it is covered by protein.
3. Antibodies specific to the protein of interest are introduced to the specimens, so the protein of interest (with DNA fragment bound to it) is immunoprecipitated.
4. We take these fragments and reverse the cross-linking between DNA and protein (purifying the DNA fragment)
5. DNA fragments are analyzed by:
PCR, next-generation DNA sequencing or microarrays

Ex: androgen binds to its receptor → receptor dimerize with other hormone bound receptor → dimer go into the nucleus (it is a transcription factor) → binds to Androgen response elements → cross-linking of receptor to DNA → fragmentation → AB addition →

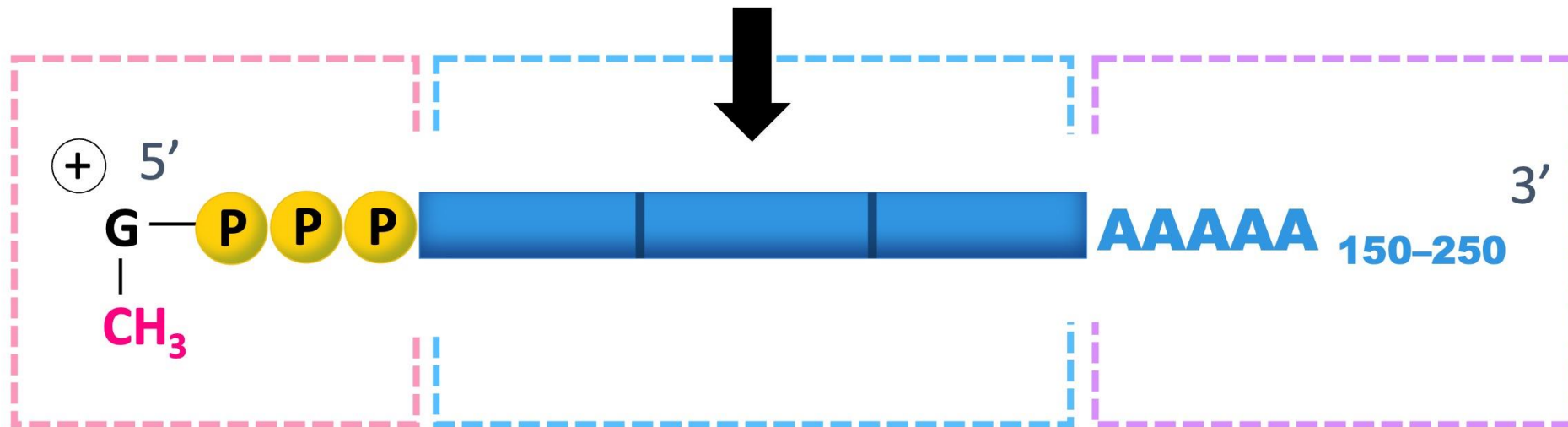




Eukaryotic RNA processing

Following Eukaryotic transcription

Processing of mRNA in eukaryotes



Capping

Splicing

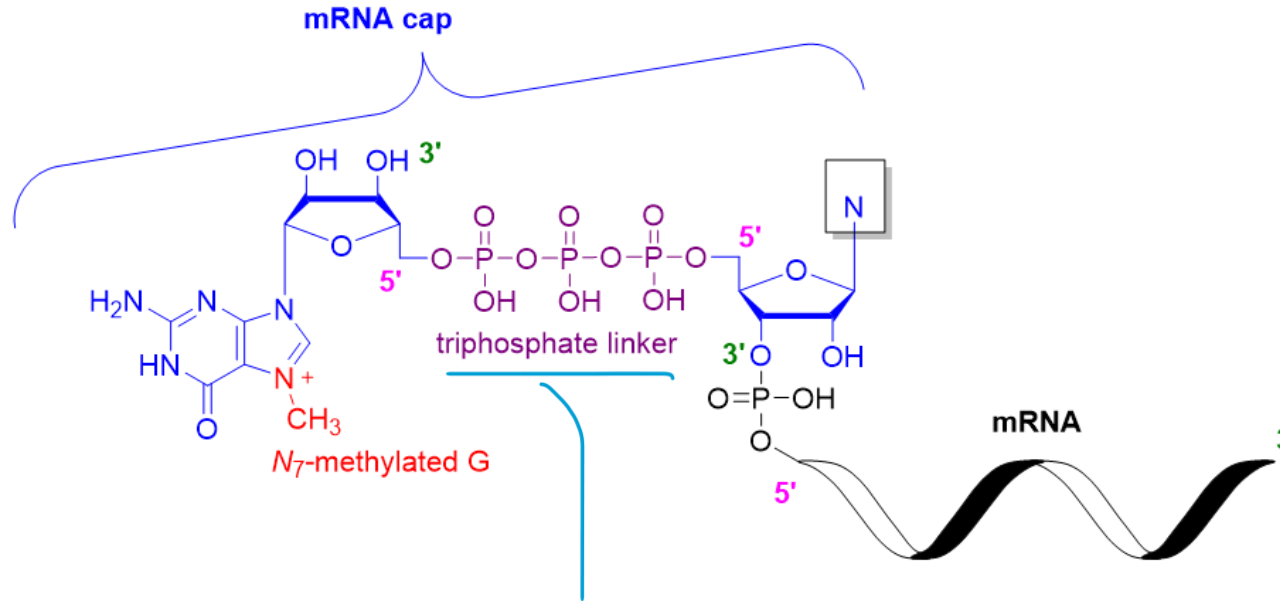
Polyadenylation

3 modifications are done to Eukaryotic RNA after transcription is done

Addition of a cap

A cap is something at the beginning of molecule

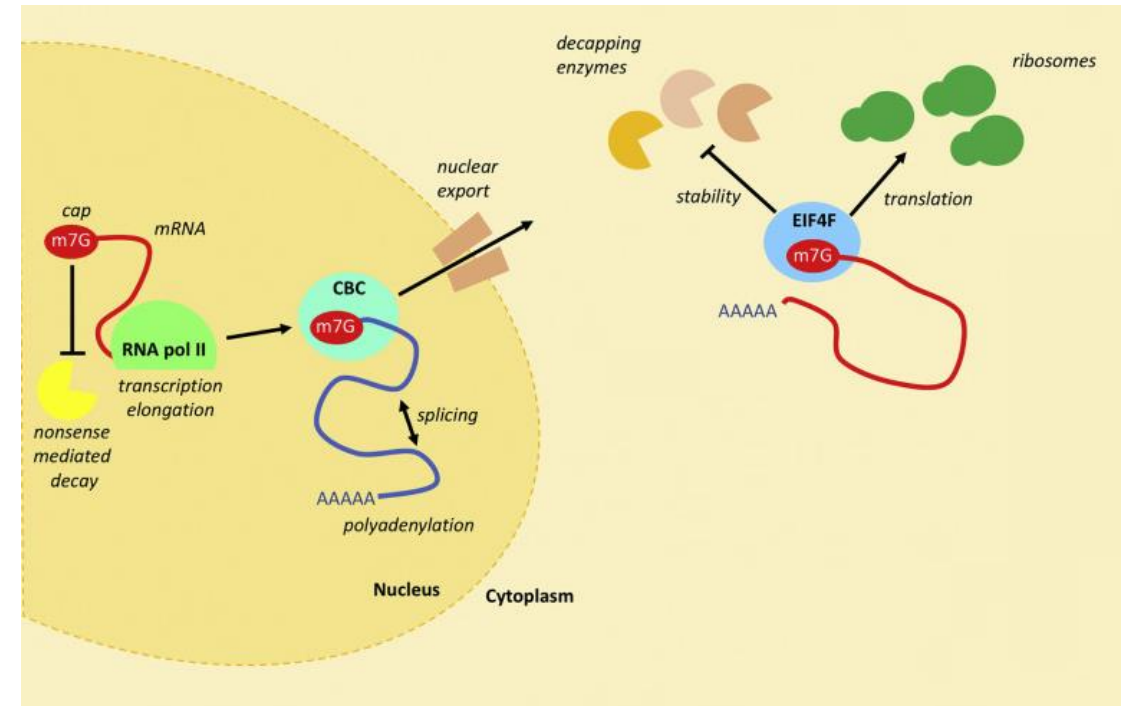
- The first modification comes as soon as RNA polymerase II has produced a few nucleotides of pre-mRNA.
- The 5' end of the new RNA molecule is modified by the addition of a "cap" that consists of a 7-methylguanosine molecule.



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

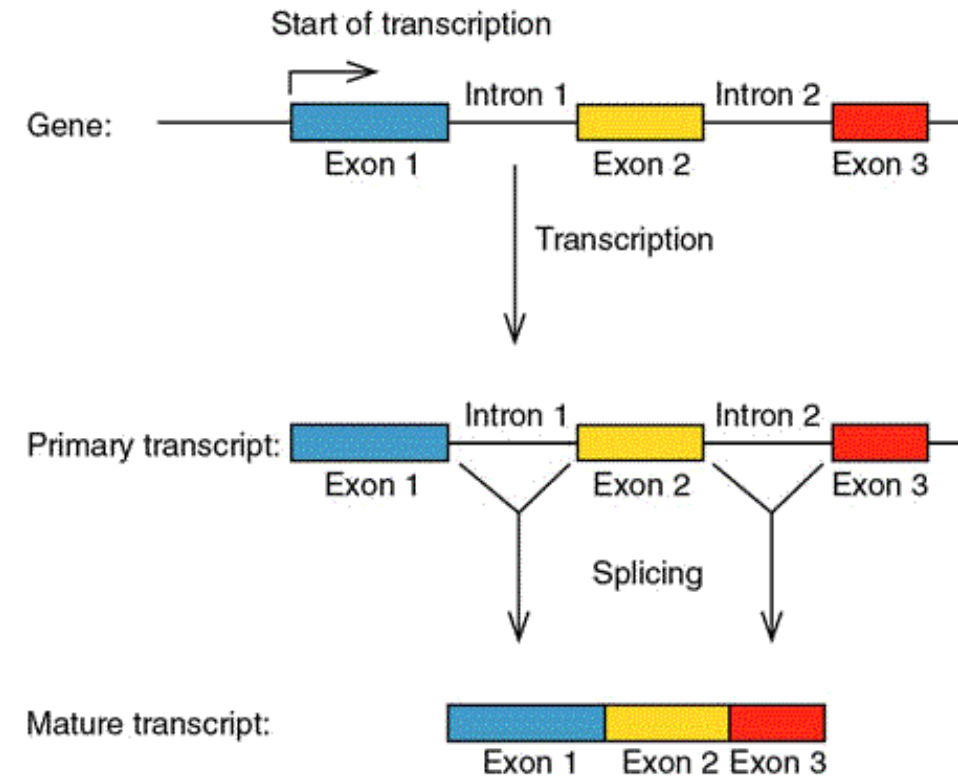
- It differentiates mRNA from other RNA molecules. **Other RNAs are not capped**
- It stabilizes the mRNA. **So, it can't be degraded by nucleases**
- It signals the 5' end of eukaryotic mRNAs.
- It recruits proteins necessary for splicing and polyadenylation.
- It helps in exporting RNA to the cytoplasm.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



Introns vs. exons and RNA splicing

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

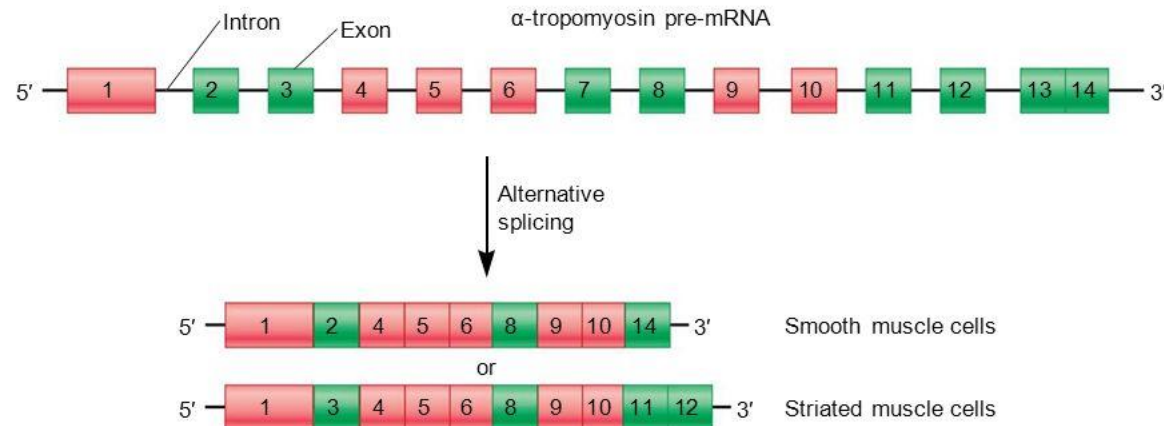
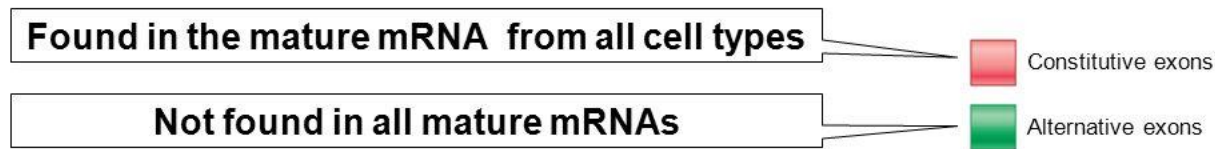
mRNA is made as primary transcript



Alternative splicing

We share the same number of protein coding genes with different organisms, so this mechanism comes to diversify genes to produce diverse proteins. Also, this explains how we have 19969 protein coding genes and 86245 protein coding transcripts (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).



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

So, order is preserved

An example is tropomyosin isoforms in different muscle types.

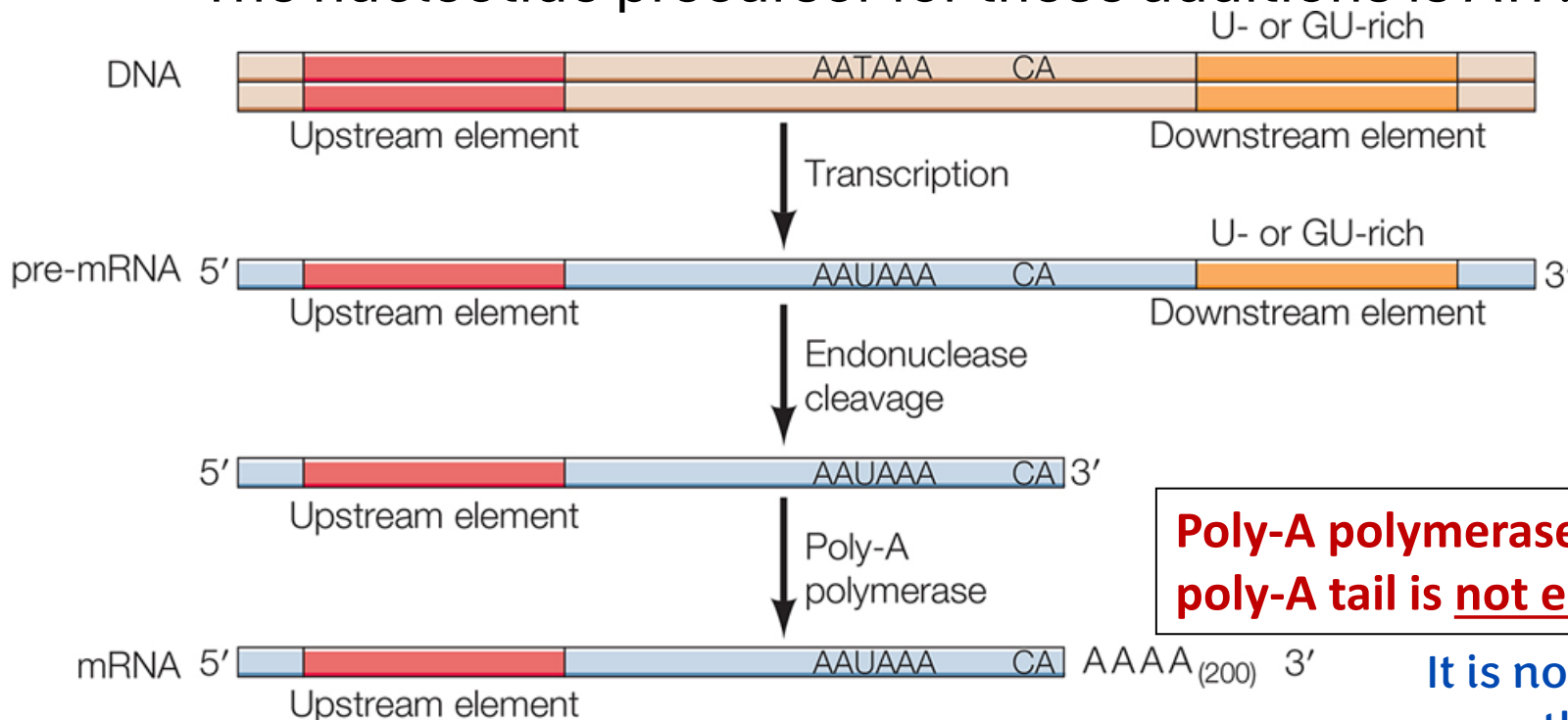
Alternatively spliced versions vary in function to meet the needs of the different cell types

- 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

Polyadenylation

Followed by CA

- A certain sequence in the mRNA (AAUAAA) 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.

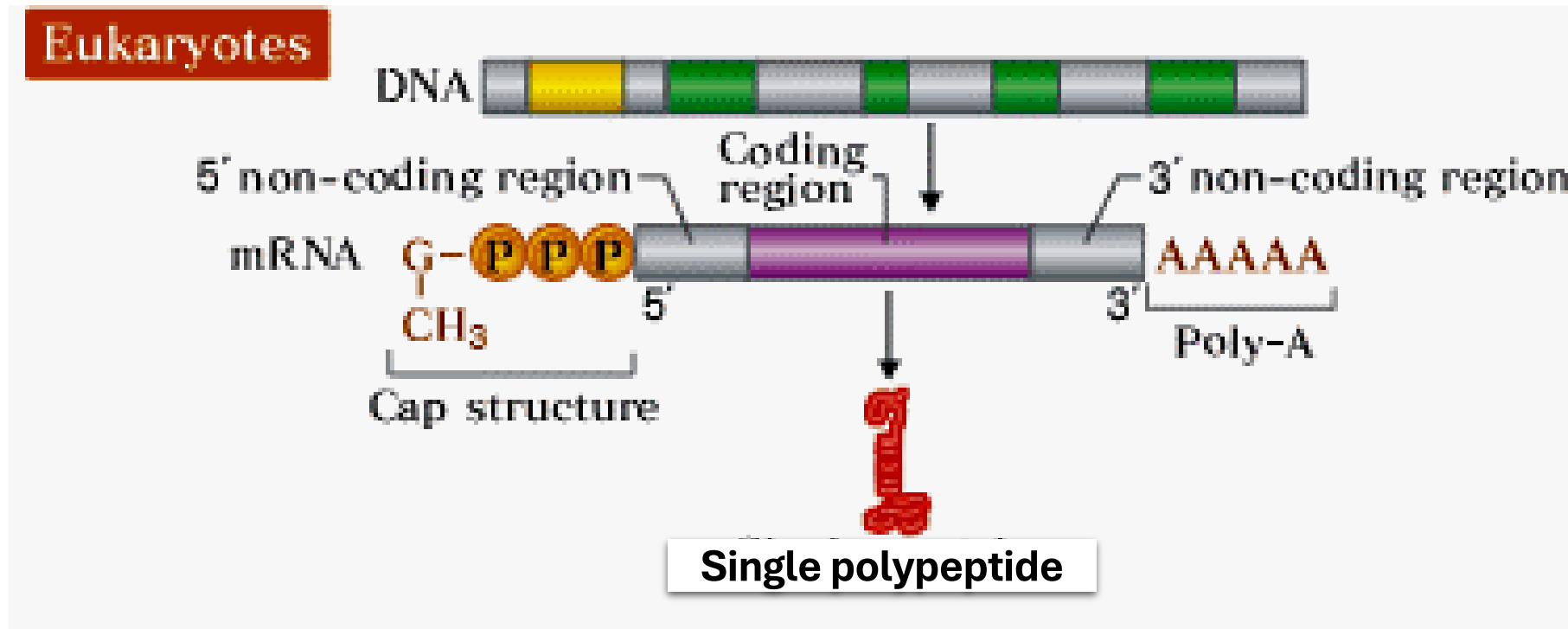
So, it dose not get degraded

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 something additional.

Eukaryotic genes

- Eukaryotic transcription units produce mRNAs that encode only one protein, thus termed **monocistronic**. Unlike prokaryotic cistron that can be polycistronic



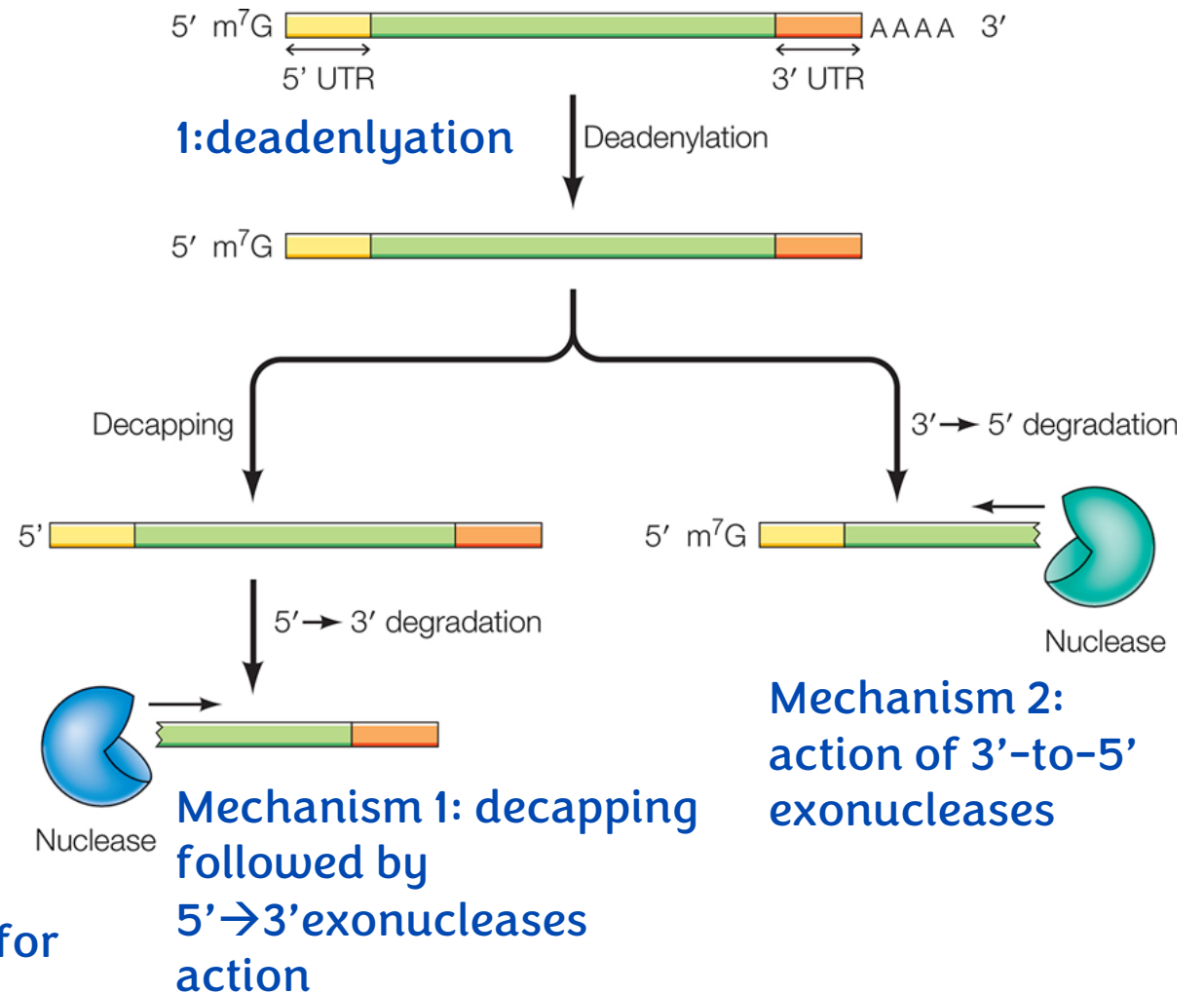
mRNA transport

- 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 AATAA 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 **may be up to 10h** 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



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			

Additional Resources:

رسالة من الفريق العلمي:

Reference Used:

(numbered in order as cited in the text)

1. the cell: a molecular approach 8th edition
part2 CH8 : 8.2&8.3 CH9: 9.2

Extra References for the Reader to Use:

1. [Enzymatic or Sonication Protocol for Chromatin Immunoprecipitation.](#)
2. https://www.youtube.com/watch?v=bHsfSteiy4E&ab_channel=NovusBiologicals (5:34-5:50) ...

قال سيدي محمد عليه أفضل الصلاة وأتم السلام بأبي هو وأمي خير الأنام: ما منكم من أحد إلا قد كُتِبَ مقعده من الجنة، ومقعده من النار قالوا: يا رسول الله أفلا نتكل على كتابنا وندع العمل؟ قال: اعملوا فكل ميسر لما خلق له، أما أهل السعادة فييسرون لعمل أهل السعادة، وأما أهل الشقاوة فييسرون لعمل أهل الشقاوة، ثم تلا قوله سبحانه: فَأَمَّا مَنْ أَعْطَى وَاتَّقَى ۝ وَصَدَّقَ بِالْحُسْنَى ۝ فَسَنُيَسِّرُهُ لِلْيُسْرَى ۝ وَأَمَّا مَنْ بَخِلَ وَاسْتَغْنَى ۝ وَكَذَّبَ بِالْحُسْنَى ۝ فَسَنُيَسِّرُهُ لِلْعُسْرَى [الليل:5-10] رواه البخاري.

فوالله ما وُضِعْتُمْ هنا عبثاً يا كرام، ولعل هذا من تيسير الله لكم لليسرى، فأعطوا الأمور حقوقها كما أمر ربي الحق، والتمسوا وجود ربنا معنا في كل صغيرة وكبيرة فإذا لم نكن نراه فإنه يرانا سبحانه وتعالى عما يصفون، ولا تيأسوا من روح الله ولا تقنطوا من رحمته، وأعلموا أن لا أمرَ كائنٍ إلا بإرادته عز وجل، فتحلوا بالأمل واصحبوه بالعمل فمن على الله اتكل لا ذاق ضيماً أو وجلاً:

فإذا العناية لاحظتك عيونها
نمّ فالمخاوف كلهنّ أمانٌ.

وأنا وقد ذكرت ما ذكرت في بدء هذا الملف، فإني ممازحٌ لا أكثر، فاصفحوا عني إن بدرَ مني الخطأ، وأدعوا لي بصلاح الحال ورضى المولى وحفظ الأهل، وللعمامة المسلمين والأمة بالنصرة والصلاح، والسلام عليكم ورحمة الله وبركاته.