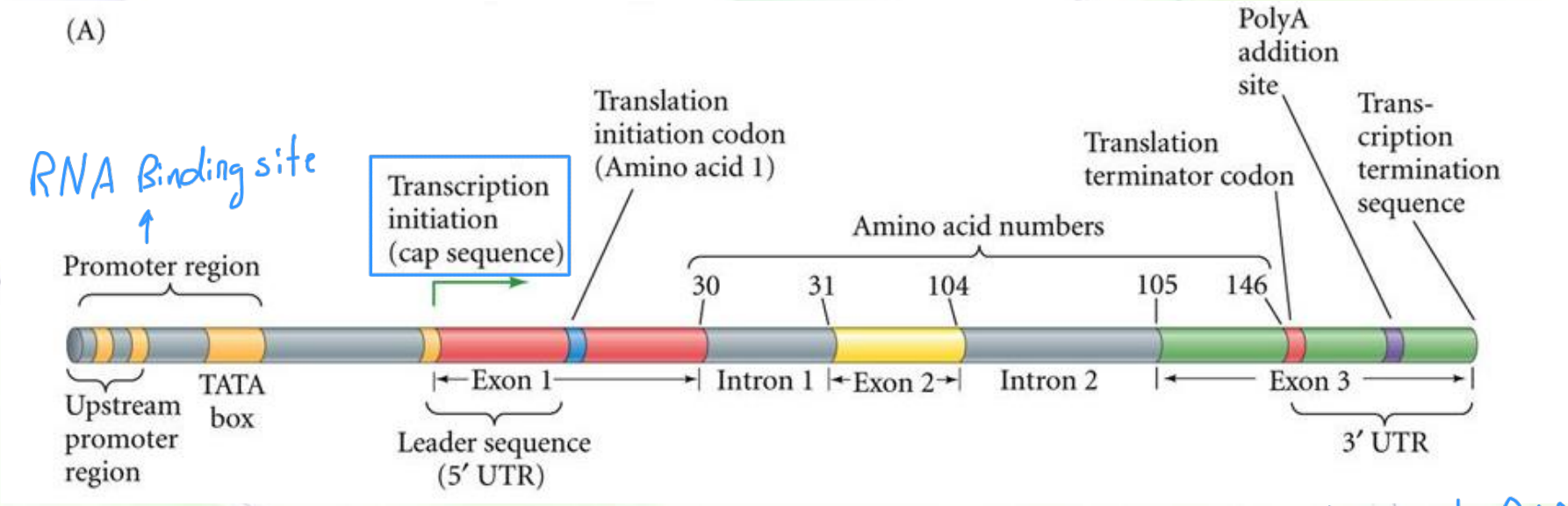
Transcription in eukaryotes

Anatomy of a eukaryotic gene



the cell must remove the introns + very proccess which will happen to RNA (on mRNA)

RNA polymerases

the bacteria has one type of RNA polymerase
the eukaryotic has at least (to this moment) 3

- 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
  - 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.

we will focus on this type

Eukaryotic RNA polymerases

in the bacteria the RNA pol has ability to open strands of DNA then transcripts then rewinding these strand togathor but the eukaryotic can't so it has general factors

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

- 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.
  - 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.

transcription factor 2 specific protein

General transcription factors

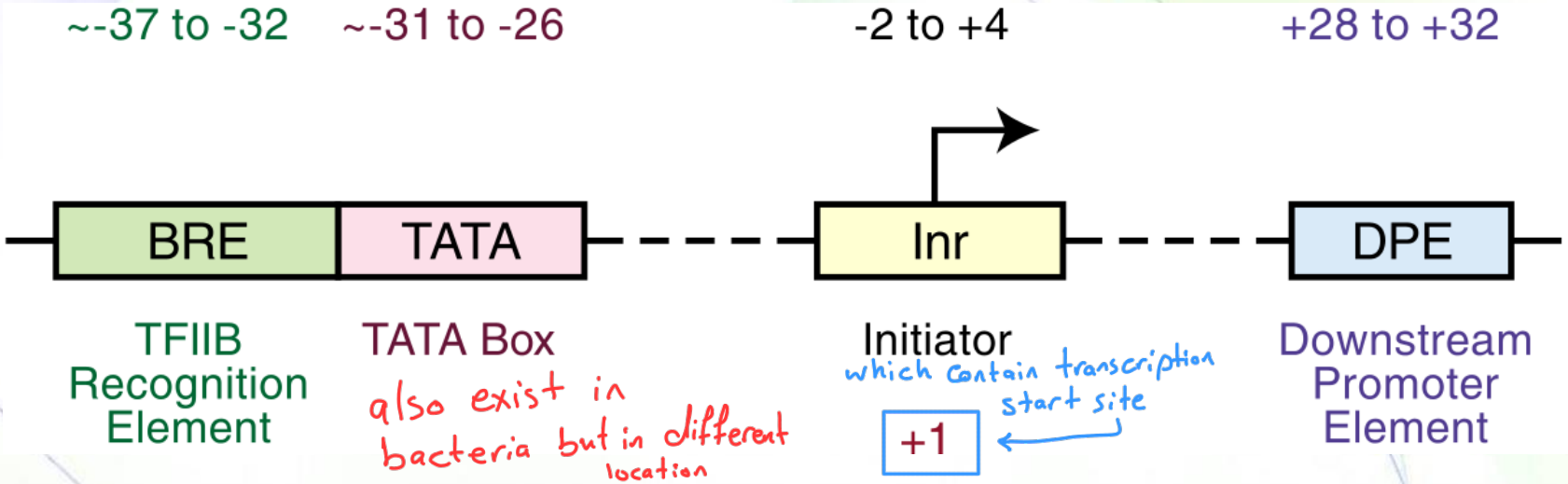
- These general transcription factors
  - help position the RNA polymerase correctly at the promoter *(like what?).* like σ subunite
  - aid in pulling apart the two strands of DNA to allow transcription to begin *(like what?).* like DnaA protein in replication
  - push the RNA polymerase forward to begin transcription. By some modifying which will talk about it.

Core components of promoters

Don't forget! in prokaryote the promoters are -10 and -35 which we called TATA Box

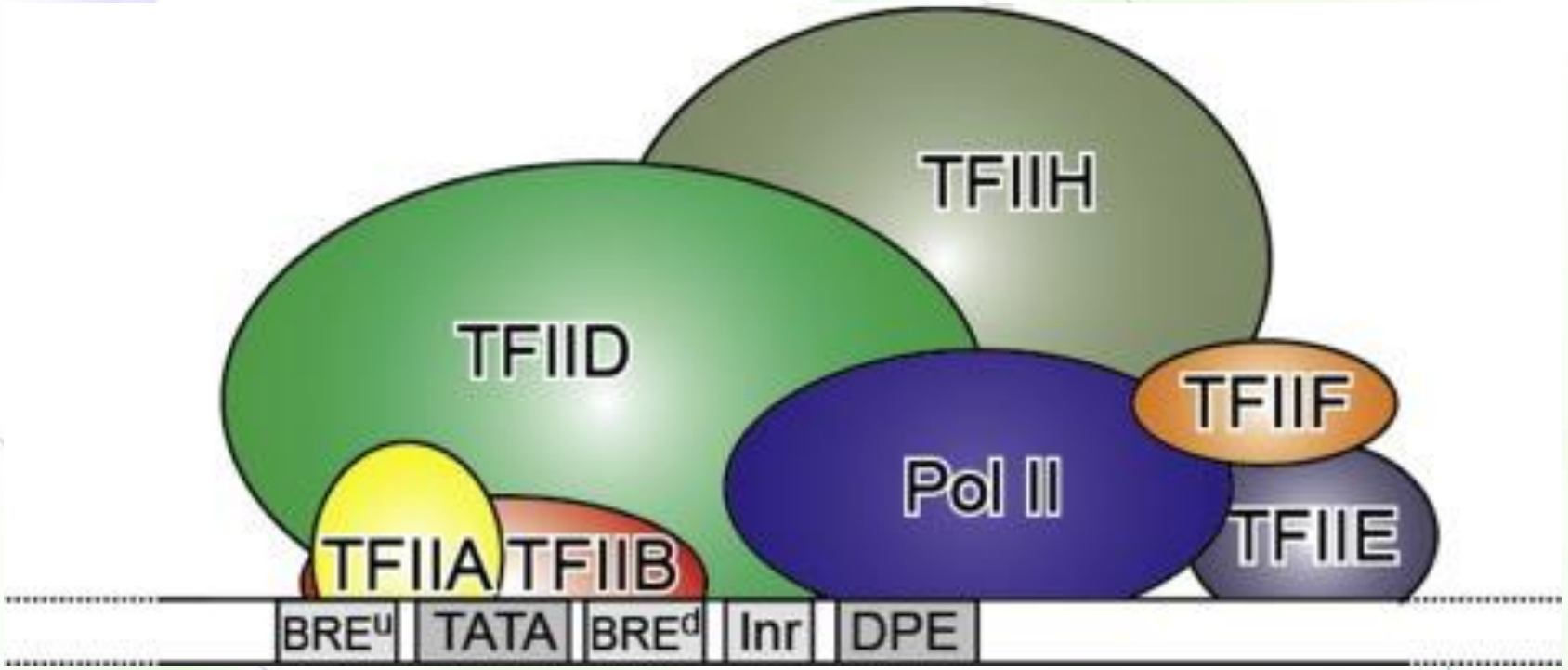
- The promoter region in eukaryotic cells is complex.

* it maybe upstream or downstream * maybe it has two promoter



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

Formation of preinitiation complex



So many proteins involved in transcription

all of these proteins is important, even the order of these proteins is important too.

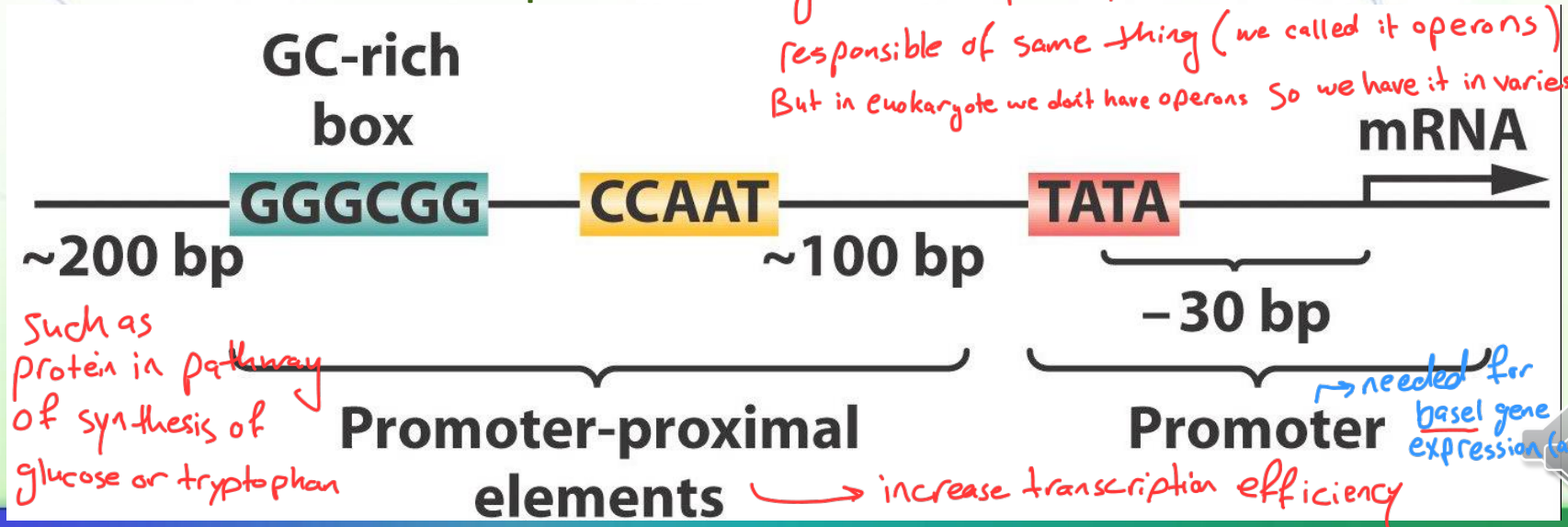
Promoter-proximal elements (with in 200 bp from TSS)

↳ example of regulatory elements : sequence in DNA in promoter which regulate transcription.

Transcription start site

- These are upstream of the core promoter region.
- 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!

* may be exist in specific genes especially that makes protein or enzyme that responsible of same thing (we called it operons)
But in eukaryote we don't have operons so we have it in varies genes

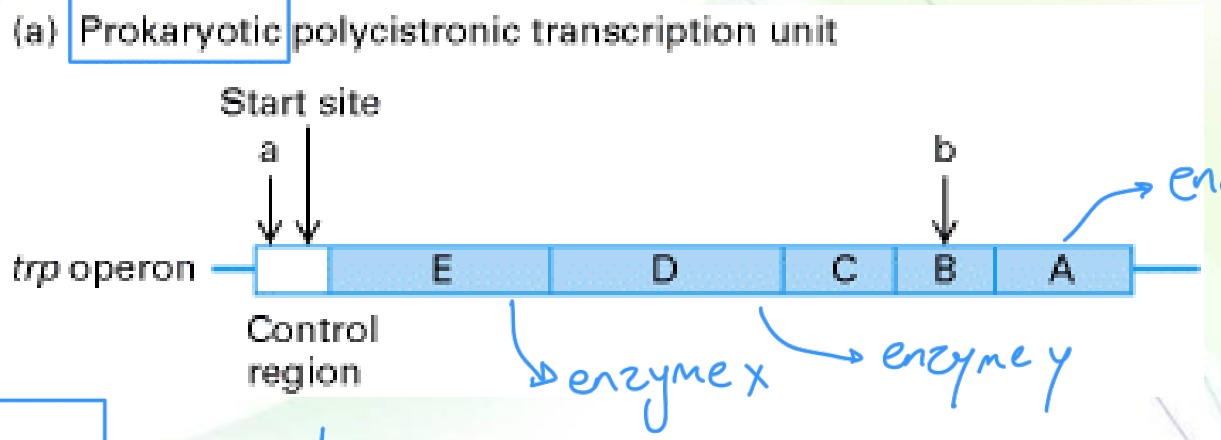


such as protein in pathway of synthesis of glucose or tryptophan

→ increase transcription efficiency

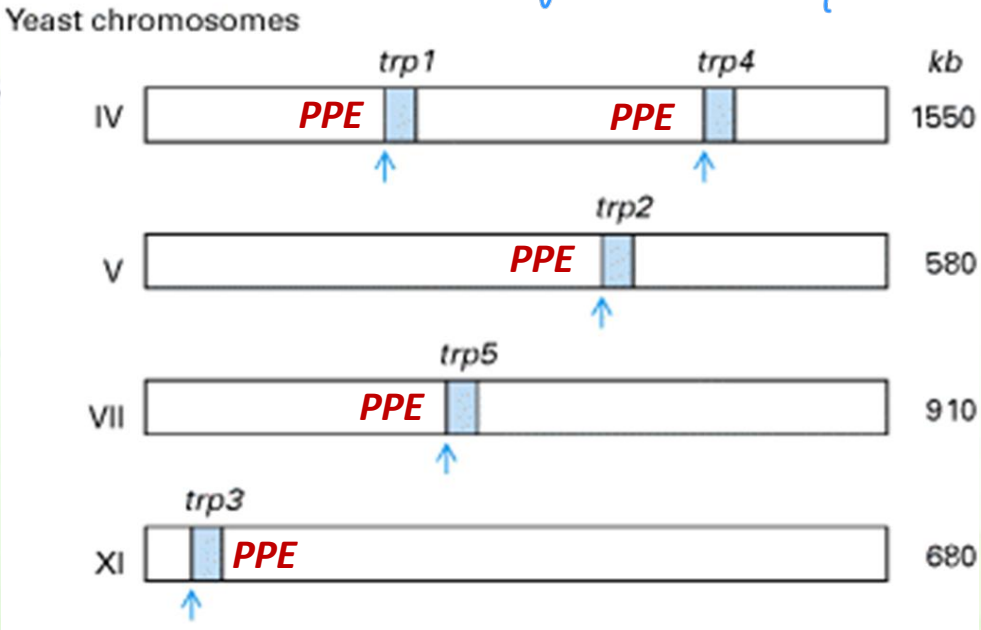
→ needed for basel gene expression (activity)

Operon vs. Proximal-promoter elements



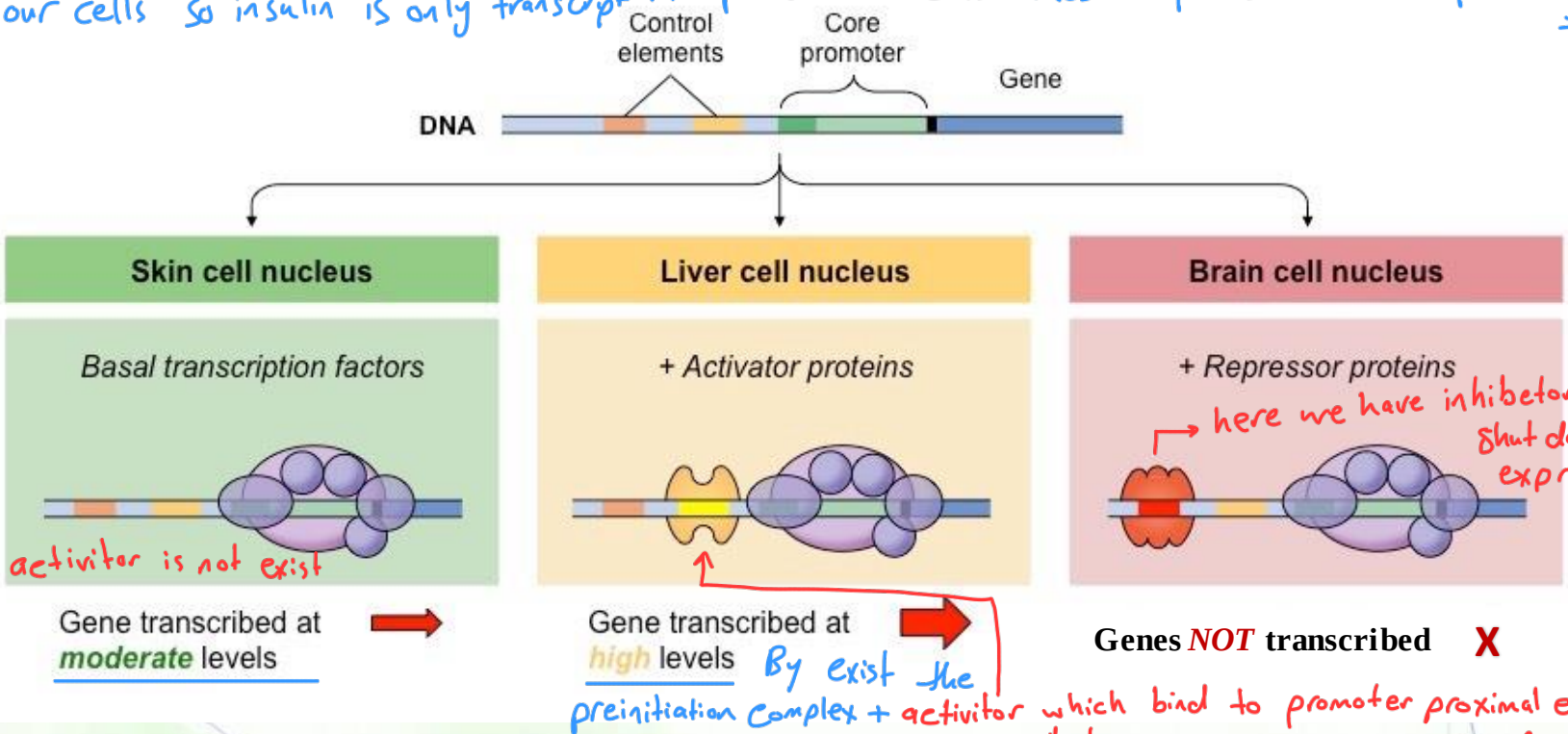
(b) Eukaryotes

but in eukaryotes the enzyme of same mechanism in defferent genes but it has the same proximal promoter element before them to transcripte in the same time



Tissue-specific transcription factors

* all our DNA is exist in all our cells but it does not transcripte in all our cells so insulin is only transcript in pancariatic cells these depends on tissue specific transcription factor



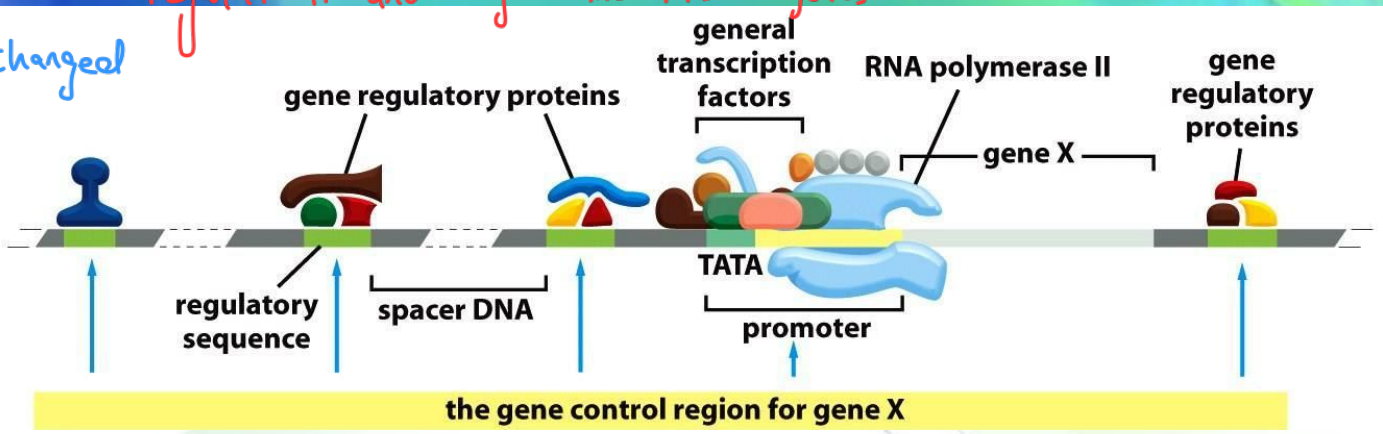
Differential expression of transcription factors (tissue-specific transcription factors) determine gene expression.

* and do not forget that some protein is transcripted in all cells.

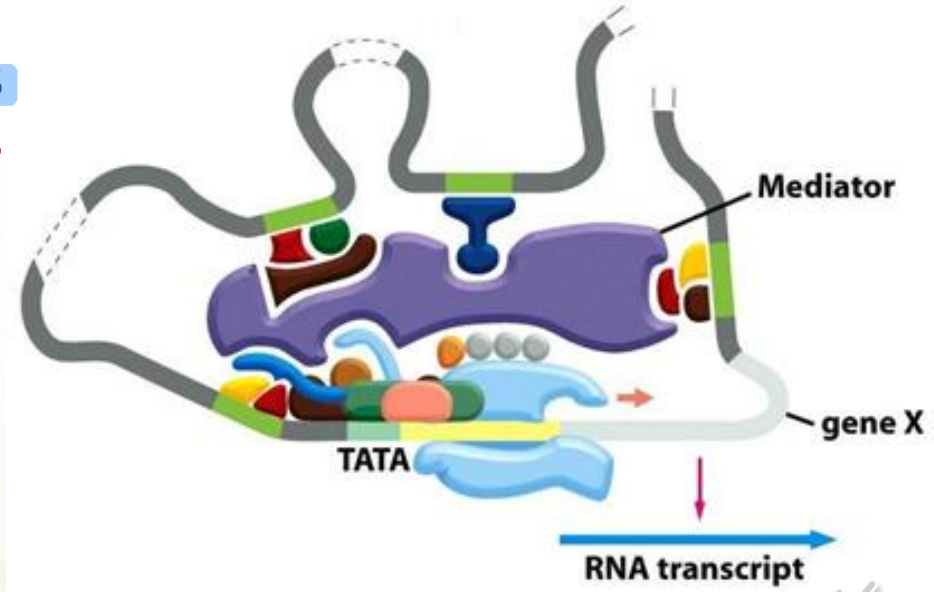
Enhancers

which maybe far from the genes that will regulate it and maybe exist in the genes

So if the gene location is changed the enhancers will keep regulate it.



- 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.

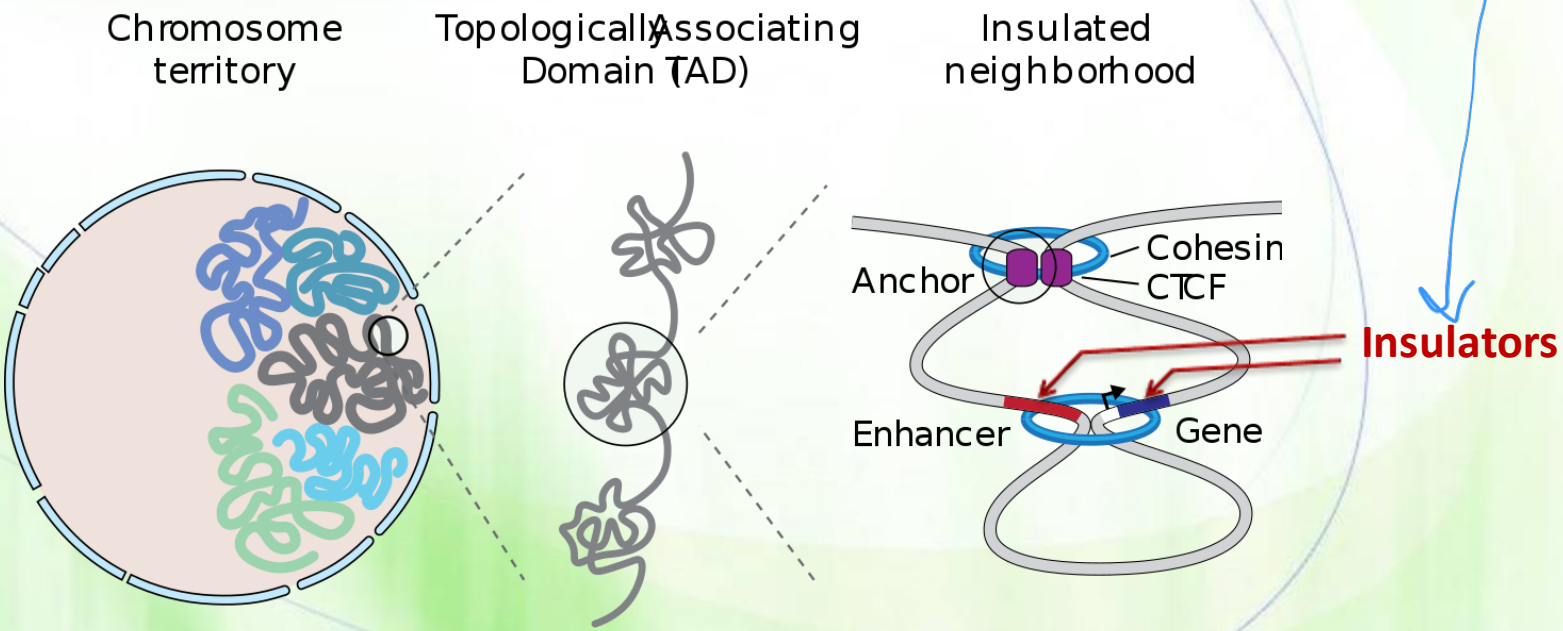


Enhancers, insulators, TADs, and CTCF

the enhancer can NOT regulate genes from other side of chromosome because the human genome is different piece making loops, these domains is saparated By insulators.

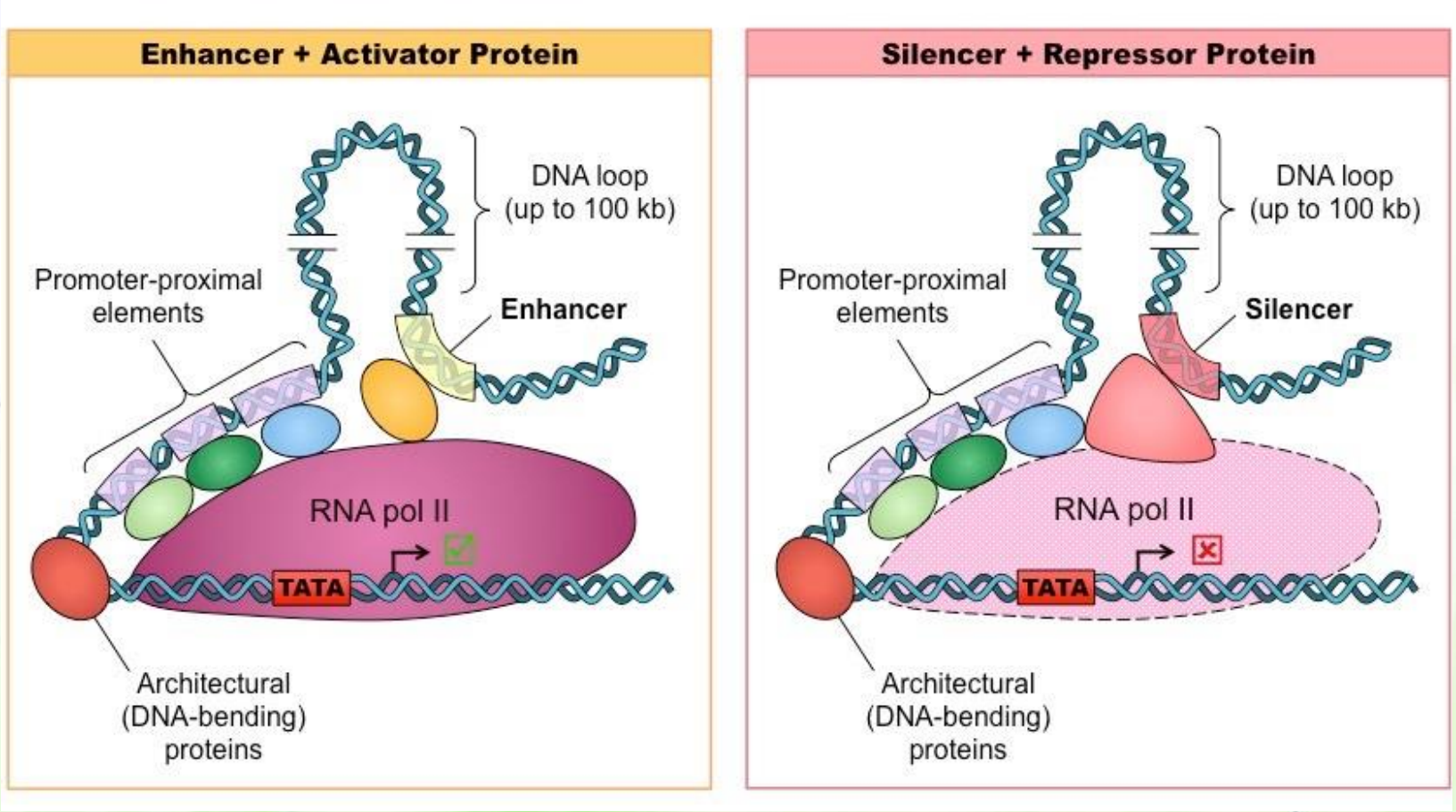
- There are 500,000 to > 1 million enhancers in the human genome (>10%).
- DNA sequences or elements known as insulators divide the genome into topologically associating domains (TADs) forming loops.
- The boundaries of the loops are stabilized by by cohesin and CTCF proteins. allowing for enhancers and promoters within TADs to interact with each other.

stabilize

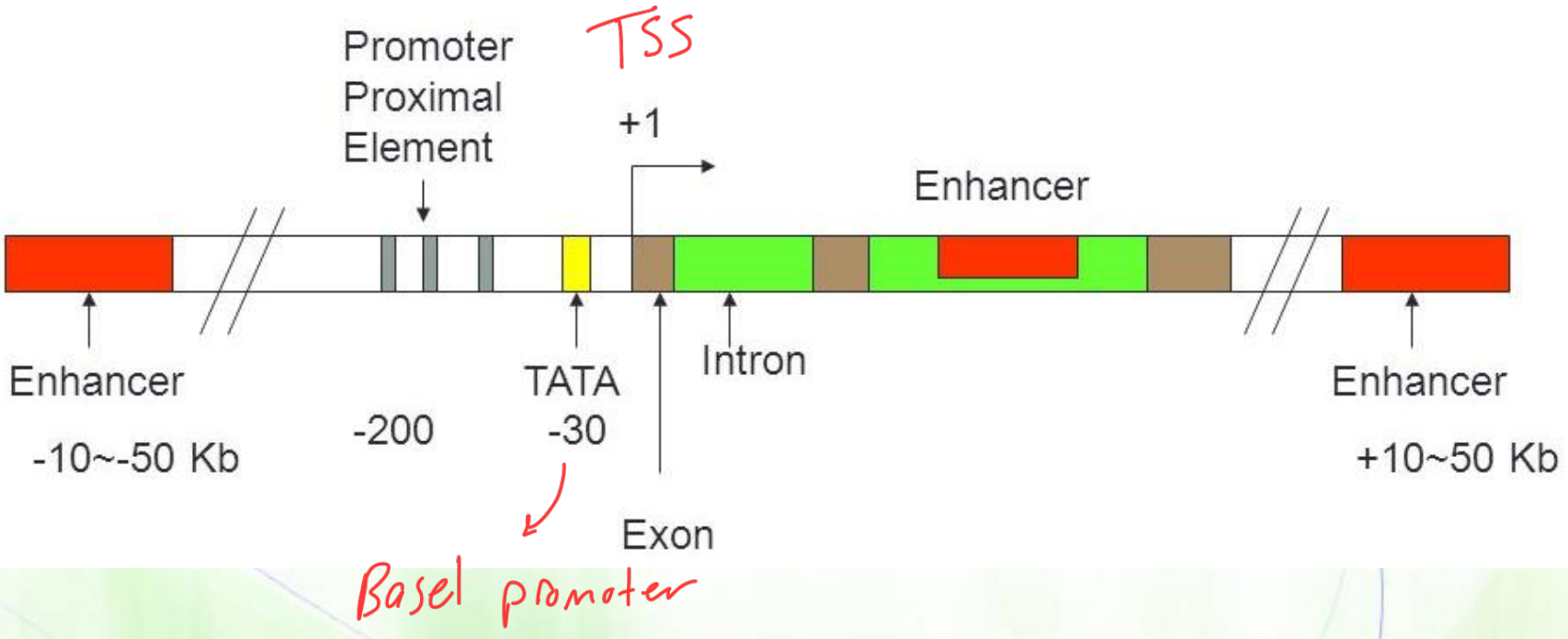


Silencers from the name it is stop the expression

- The opposite of enhancers.

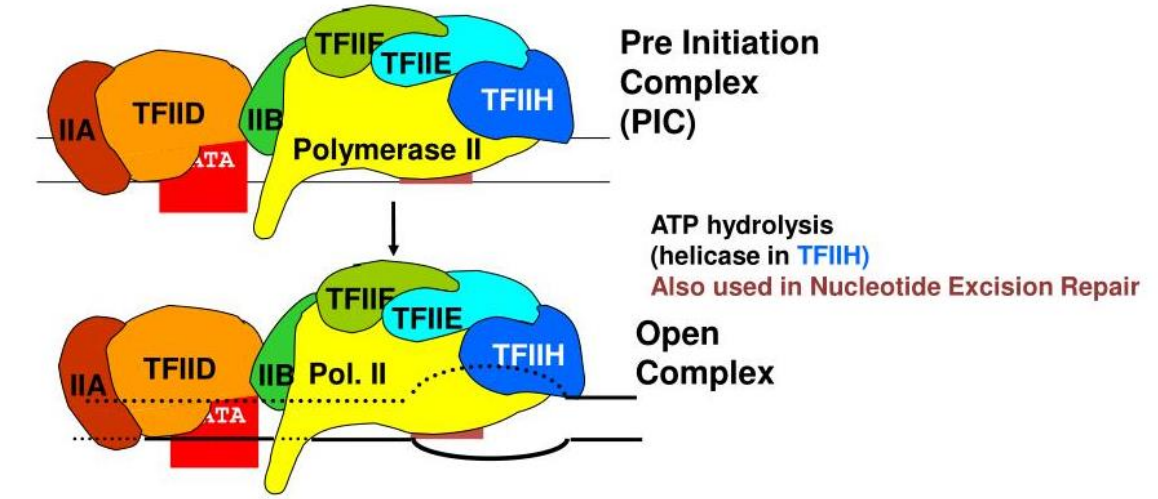


A hypothetical regulatory sequence of a mammalian gene



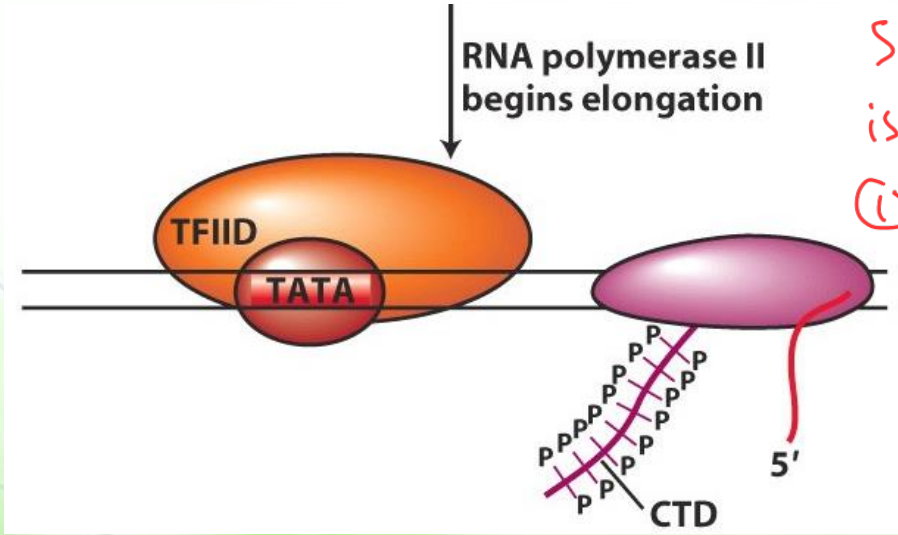
Mechanism of transcription (initiation) first Step

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



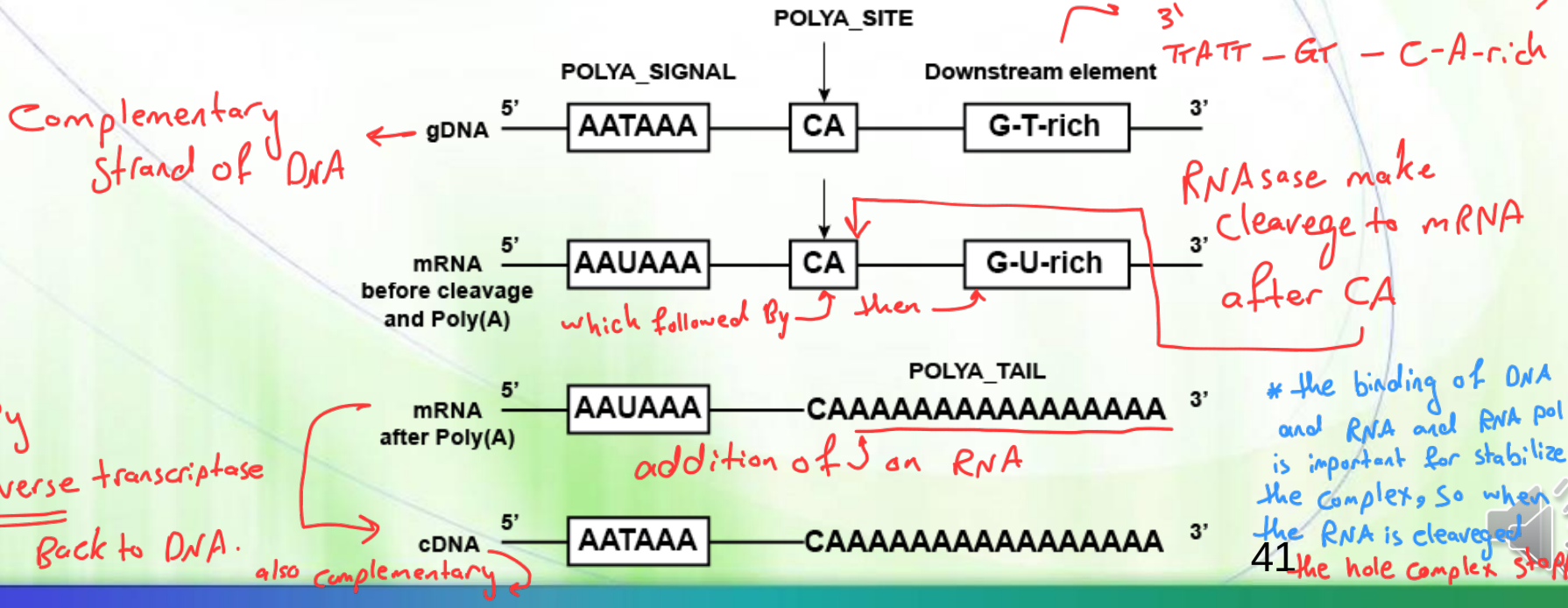
Mechanism of transcription (elongation)

- 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 subunits.



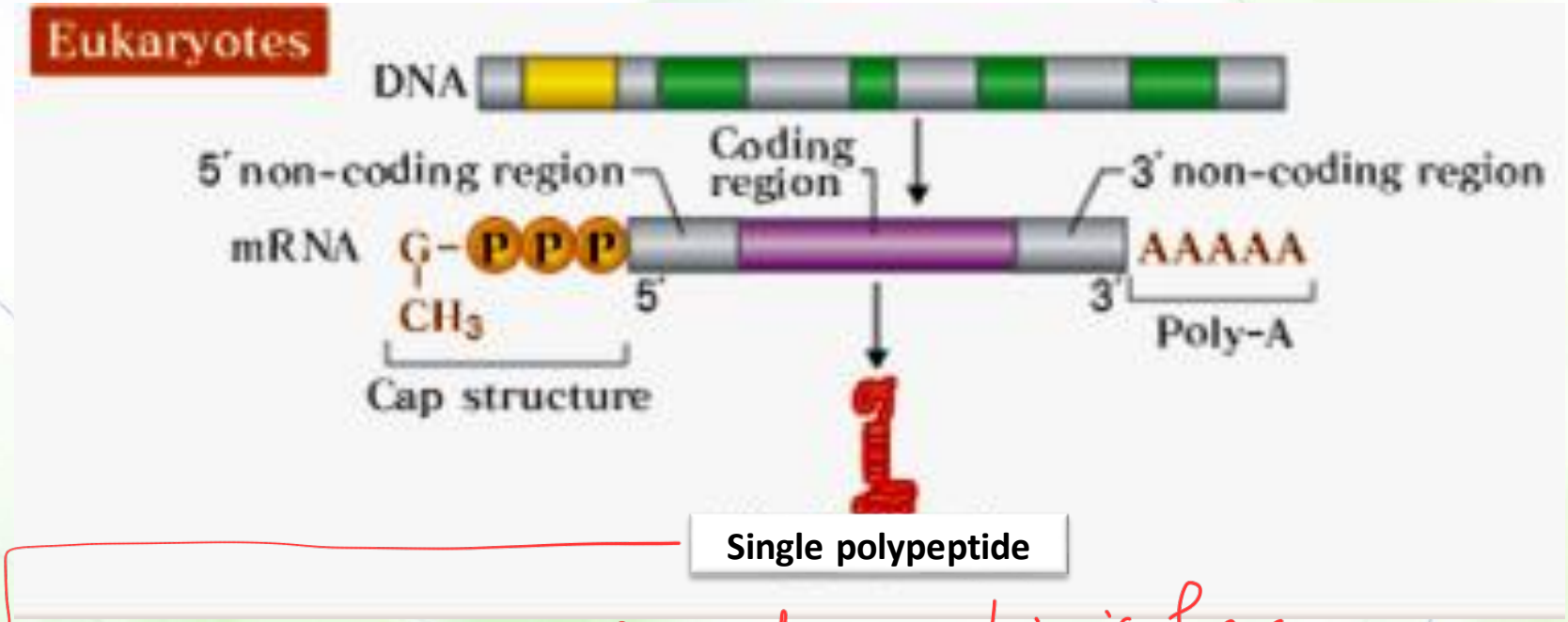
Mechanism of transcription (termination)

- 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?
- Termination is coupled to the process that cleaves and polyadenylates the 3'-end of the transcript.



Eukaryotic genes

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



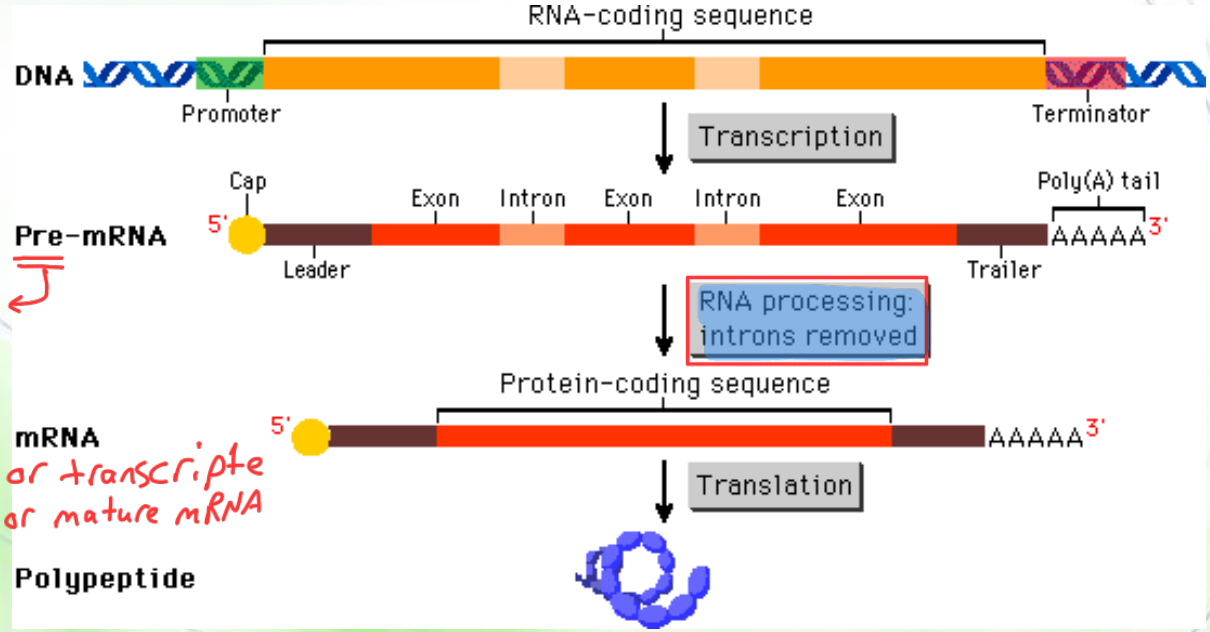
→ maybe modified and produce protein isoforms or glycosidation or phosphorylation or lipidation or clearage to smaller peptides

Introns vs. exons

↳ non-coding genes

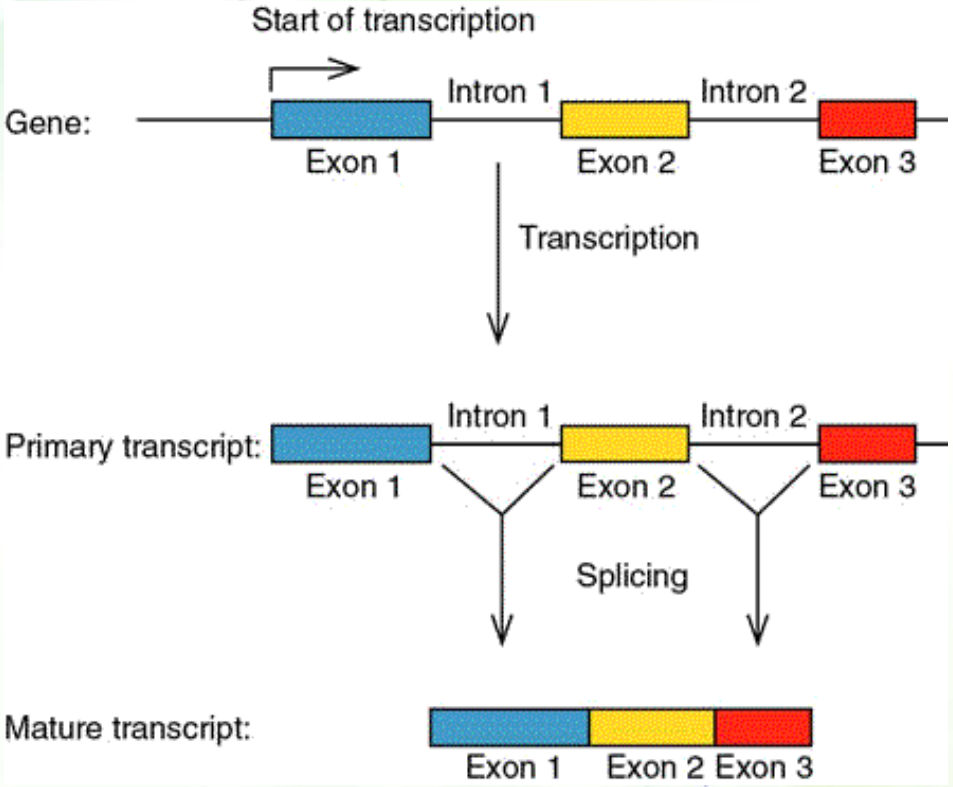
- The genomes of eukaryotic cells contain specific DNA sequences that do not code for proteins known as **introns**.
  - The protein-coding regions are known as exons.
- When RNA is synthesized, the RNA molecule contains both introns and exons and is known as pre-mRNA.

has introns and exons



RNA splicing : removing of introns and connecting exons to each other.

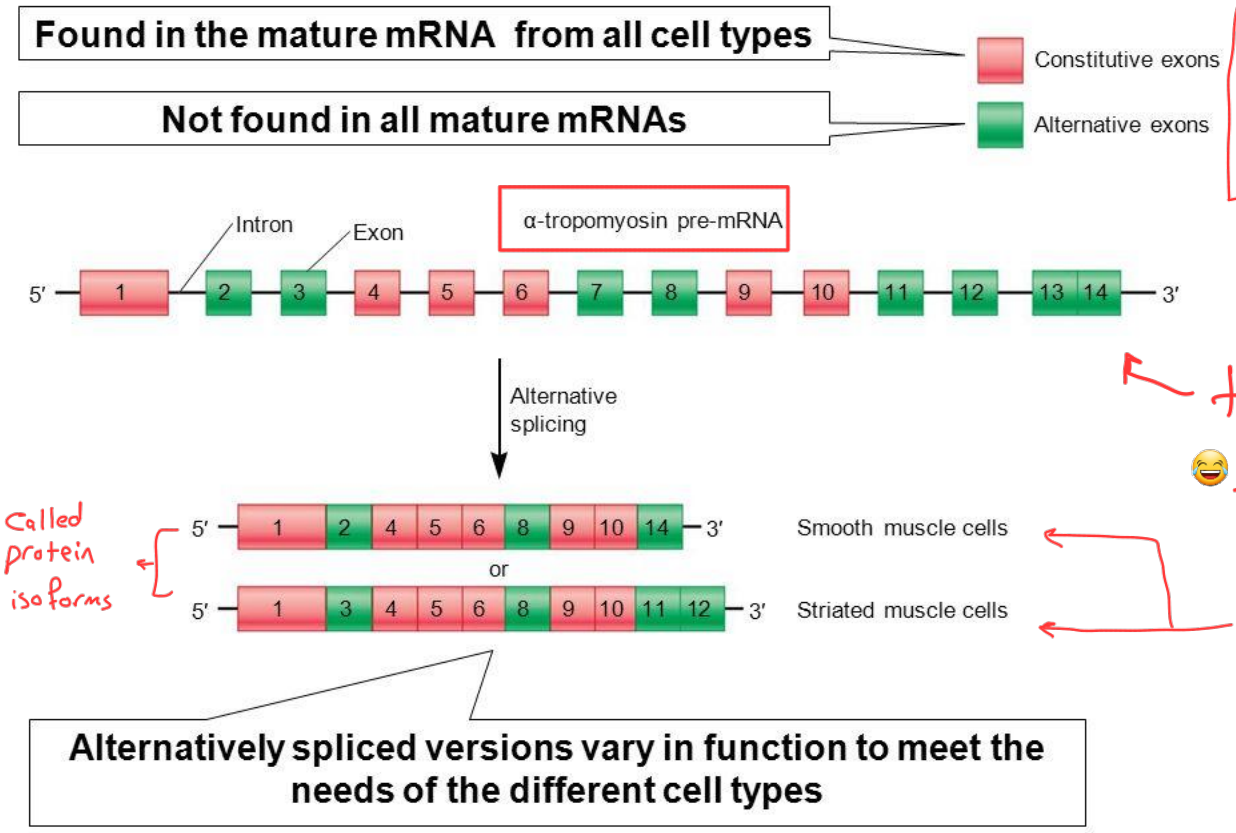
- The intron sequences are removed from the newly synthesized RNA through the process of RNA splicing.
- Now the RNA molecule is known as mRNA (mature transcript).



Alternative splicing

so the cell choose the exon that she want

- 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).



the location of exons is static so the cell determine only if it need the exon or not.
So exon 9 will never come after 10

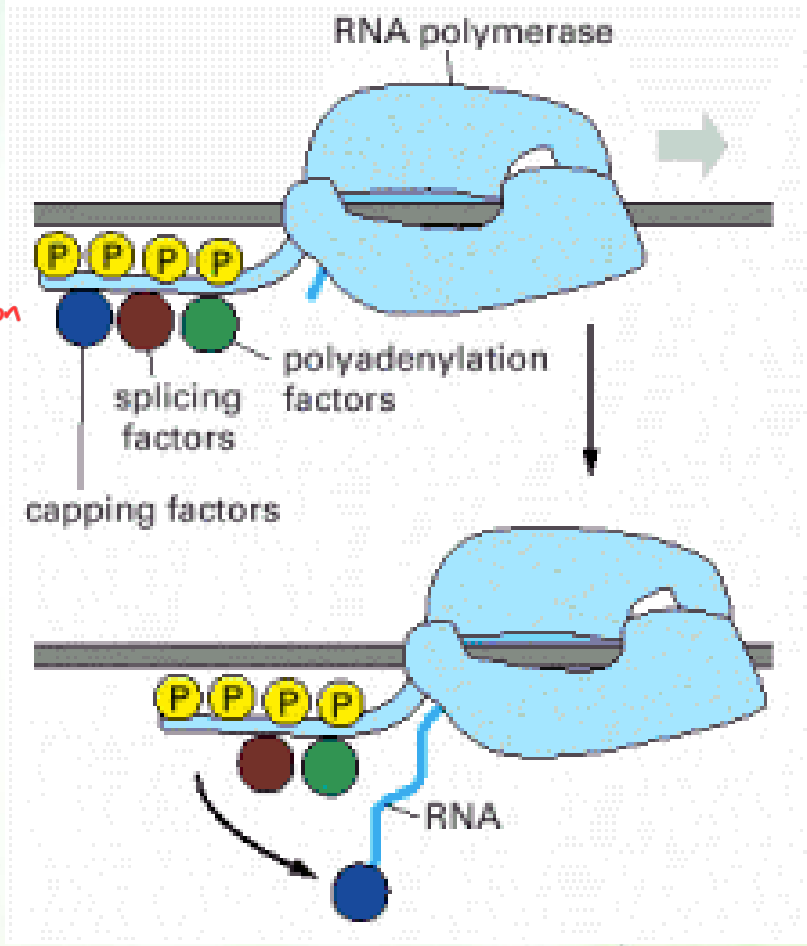
Note: Exons that are 3' to another exon are never placed 5' to it after splicing.

tropomyosin (actin Binding protein)
😂 ما كفت تنساه بالهستو
So it is responsible for contraction even it diffrent in mechanism due different in splicing

Processing of mRNA in eukaryotes

↳ is contained in RNA transcription process

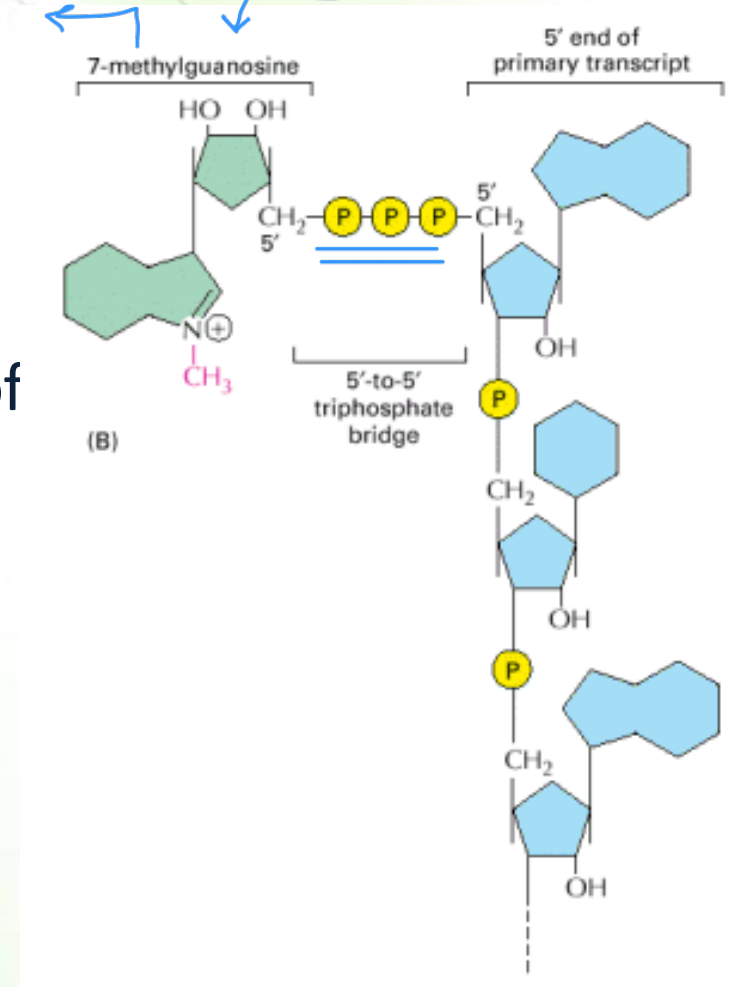
- mRNA is processed and modified extensively
  - Capping first factors work
  - Splicing work after the end of transcription
  - Polyadenylation cleavage of RNA
- Some of these processing proteins are associated with the tail of RNA polymerase II.
- These proteins jump from the polymerase tail onto the RNA molecule as it appears.



Addition of a cap

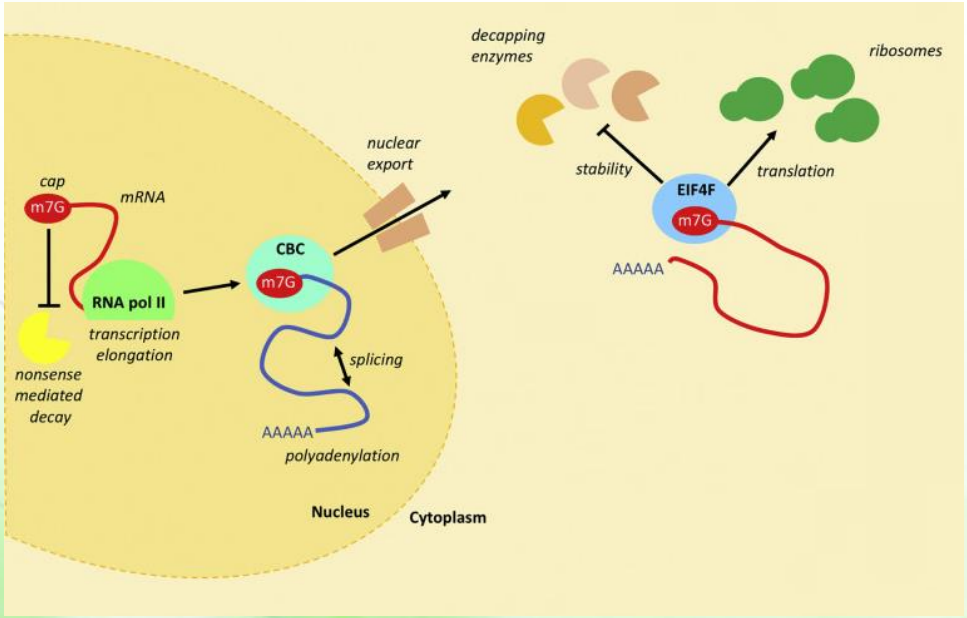
- As soon as RNA polymerase II has produced about ~25 nucleotides of pre-mRNA, the 5' end of the new RNA molecule is modified by addition of a "cap" that consists of GTP in reverse orientation.
  - 5’ to 5’ instead of 5’ to 3’.

all heads of nucleosides is for up except the cap to the down



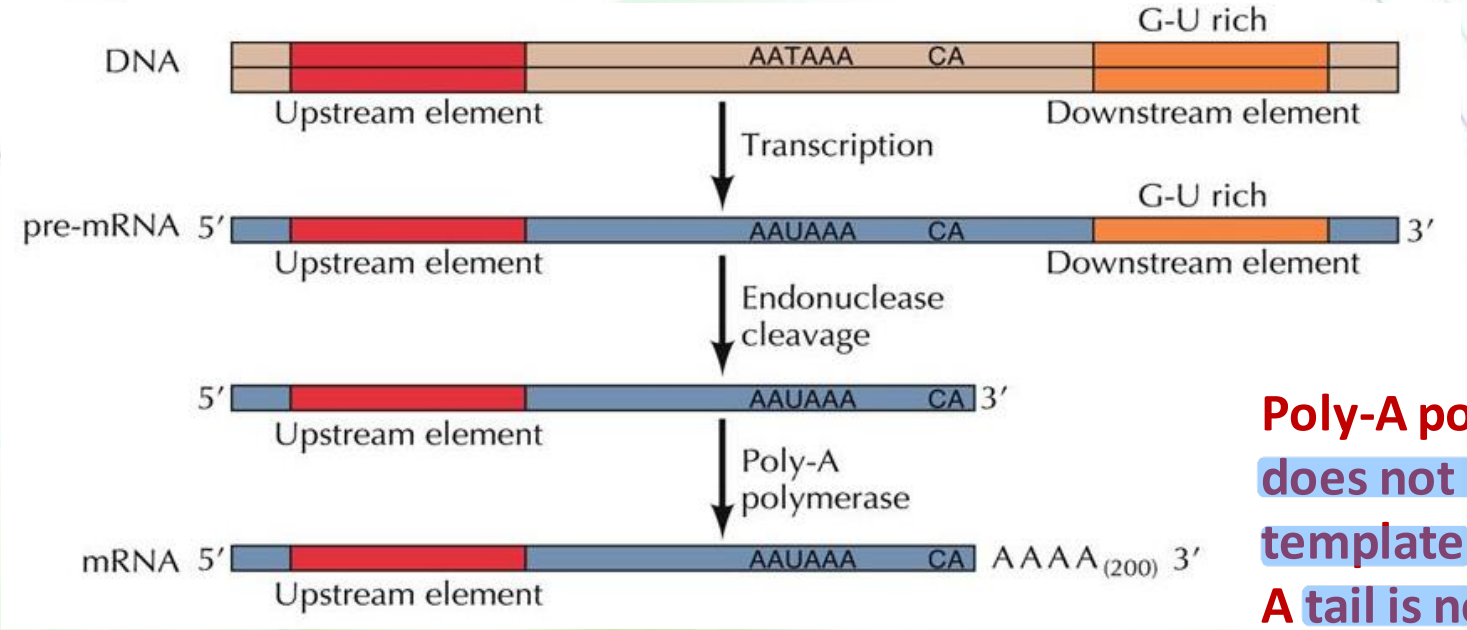
Importance of capping

- It stabilizes the mRNA.
- It signals the 5’ end of eukaryotic mRNAs.
  - This helps the cell to distinguish mRNAs from the other types of RNA molecules, which are uncapped.
- It recruits proteins necessary for splicing and polydenylation.
- It helps in exporting RNA to the cytoplasm.
- It helps in the translation of mRNAs to proteins. to read it from the cap



Polyadenylation

- A certain sequence in the mRNA (AAUAAA) in the 3’ ends of mRNAs is recognized by enzymes that cleave it.
- Poly-A polymerase then adds ~200 A nucleotides to the 3’ end.
  - The nucleotide precursor for these additions is ATP.



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

Significance of polyadenylation

- It helps in transporting mRNA from the nucleus to the cytosol.
- It helps in translation.
- It stabilizes mRNA. So it can be degredaded

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. and regulated

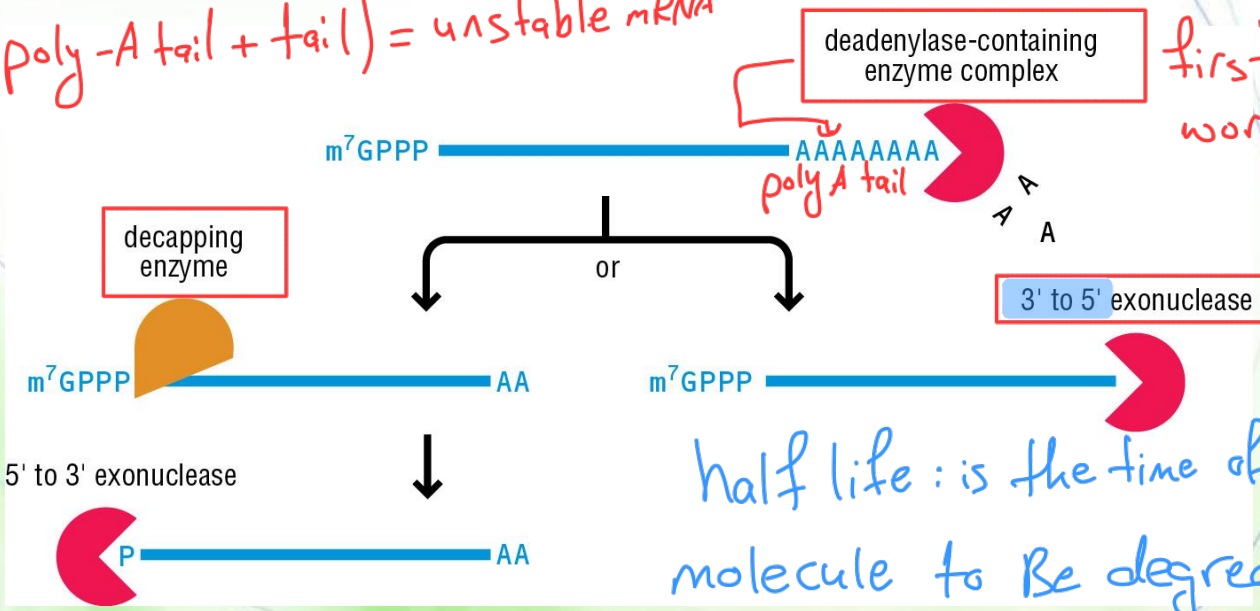
- Defective mRNA molecules like interrupted RNA, mRNA with inaccurate splicing, and so on, are not transported outside the nucleus.

Degradation of mRNAs

- 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).
- Degradation of eukaryotic mRNA is ainitiated 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.

from the terminatitions

mRNA - (poly-A tail + tail) = unstable mRNA



half life : is the time of half molecule to Be degredaded

الموسم
الرابع عشر

امسح الكود للتسجيل

أكاديمية
ZAD ACADEMY

لتعليم العلم الشرعي وهو أهم العلوم وأشرفها

التسجيل
مازال متاحًا

ما لا يسع المسلم جهله

- مشاركة تفاعلية مجانية
- منصة الكترونية احترافية عصرية
- مرونة في المواعيد وعدم الحاجة للتفرغ
- يُبث عبر قناة زاد الفضائية

سجل الآن وتَزَوَّدْ من العلم

register.zad-academy.com

AcademyZAD zadacademy

A phenomenon in eukaryotes

ظاهرة

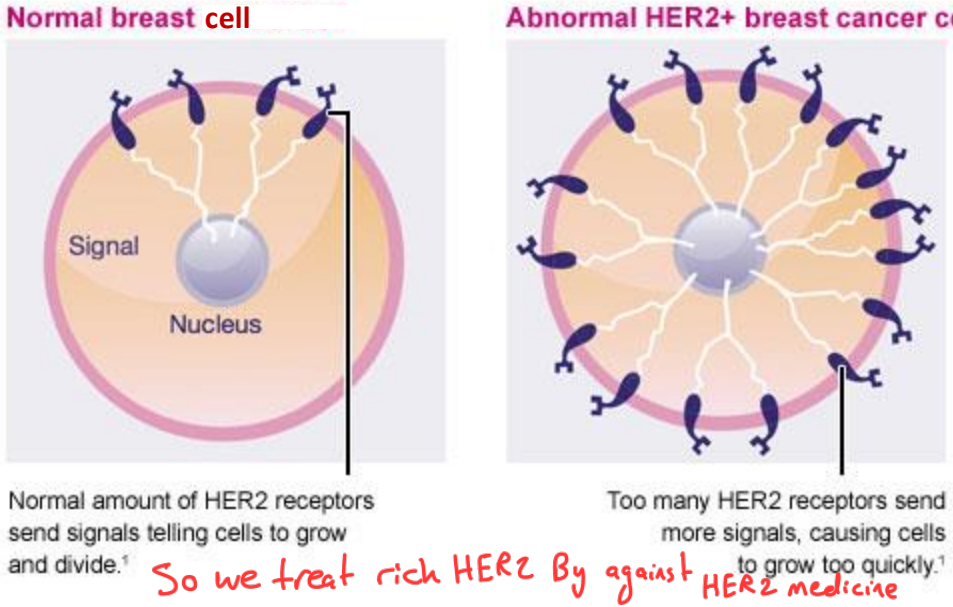
we use it to increase protein number

Gene amplification

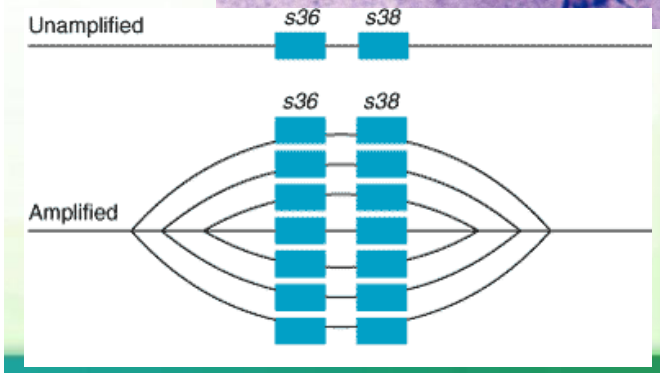
so if we had 1 gene now we have 10 genes

more genes = more mRNA = more protein

- It is an increase in copy number of a restricted region of a chromosome increasing the is the quantity of DNA in these regions.
- It is a mechanism that cancer cells use to develop resistance from methotrexate whereby the target gene, dihydrofolate reductase, is amplified. in cancer cells so the methotrexate can not inhibite this enzyme
- It is also a mechanism by which breast tumor cells progress and become more aggressive whereby they amplify the human epidermal growth factor receptor 2 (HER2), which stimulates cell growth. By any small factor.



So we treat rich HER2 By against HER2 medicine



* a Simple gene is the gene which has Single promoter and does not do splicing which transcripte to Single mature mRNA ⇒ Single polypeptid by translation

* So we have about 20k genes, How we get more than 80k mRNA ?

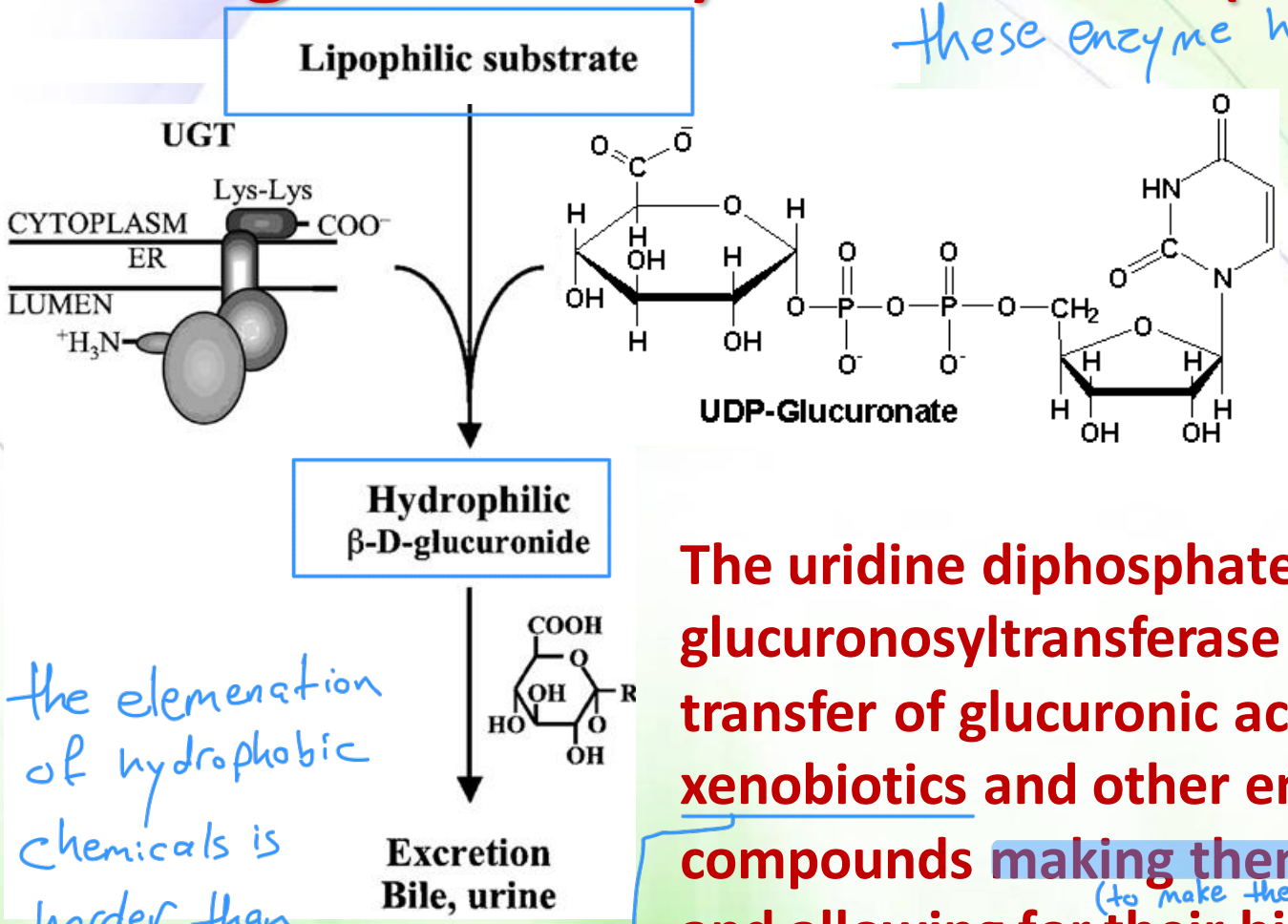
① By splicing ② Some phenomenon as

Another phenomenon:
Multiple forms of a single exon

An example of alternative splicing:

UDP-glucuronosyltransferase (UGT)

these enzyme has many substrates (targets)



the elemenation of hydrophobic chemicals is harder than hydrophilic

The uridine diphosphate glucuronosyltransferase (UGT) enzymes transfer of glucuronic acid onto xenobiotics and other endogenous compounds making them water soluble (to make them hydrophilic) **and allowing for their biliary or renal elimination.**

the chemicals that can inter our body like medicines.

It has many substrates with different structures

this Slide not for memorizing

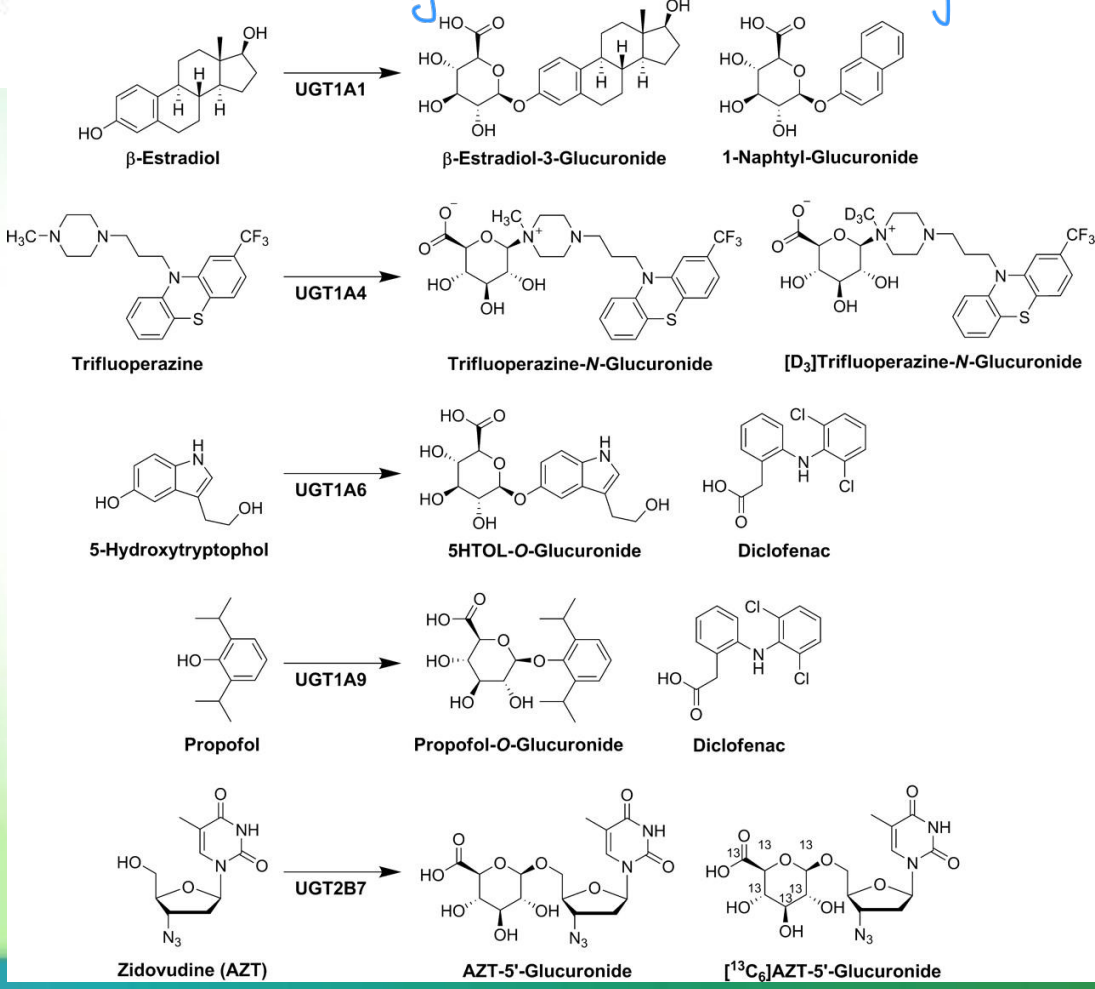
Lipophilic substrate

Therapeutic drugs	Biliary acids
Carcinogens	Steroïds
Environmental toxicants	Retinoic acids
Dietary constituents	Fatty acids
Bilirubin	

Substrates
Etoposide
Genistein
Tamoxifen
PCBs
heterocyclic amines
Benzo[a]phrene
Nicotine
Raloxifene

It is a family of enzymes that is responsible for the glucuronidation of hundreds of compounds, including hormones, flavonoids and environmental mutagens.

So many substrate for the enzyme



and reactions are catalyzed in different tissues

the UGT enzyme is tissue specific but substrate not specific

Substrates	Place of reaction
Etoposide	Biliary tissue, colon, intestine, liver, stomach
Genistein	Biliary tissue, colon, liver, stomach
Tamoxifen	Biliary tissue, colon, intestine, liver
PCBs	Biliary tissue, brain, colon, kidney, larynx, liver, lung, stomach
Heterocyclic amines	Esophagus, intestine, kidney, larynx
Benzo[a]phrene	Colon, esophagus, intestine, kidney, larynx
Nicotine	Breast, colon, esophagus, liver, kidney, ovary, prostate, skin, testis
Raloxifene	Biliary tissue, colon, esophagus, intestine, orolaryngeal tissue, stomach

Get this concept, first...

So the enzyme do the same job but with different substrates

adding the Glucoronate to the substrates

One drill, many flutes

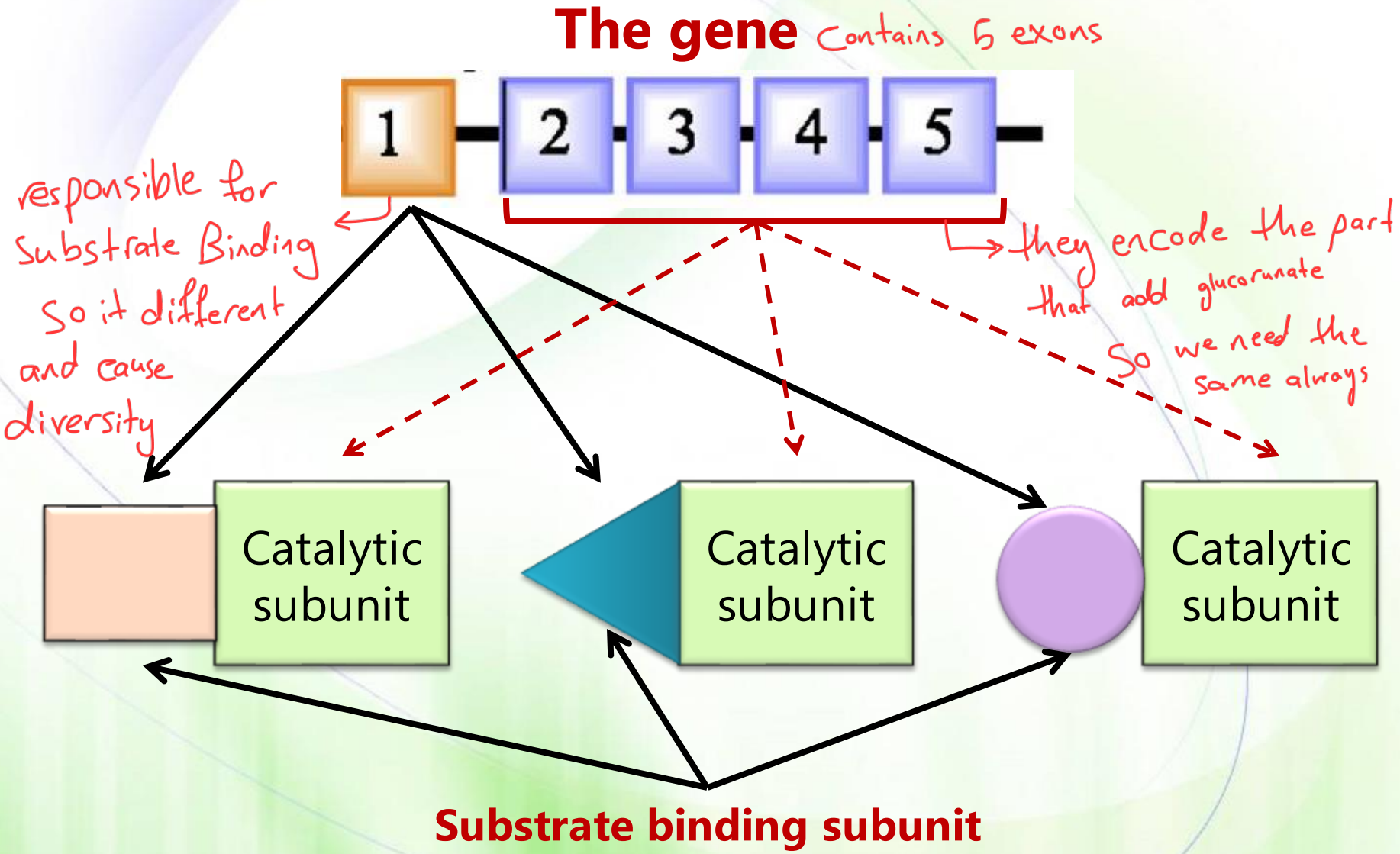
the same function but different (رِيشْن)

One head, many hats

Same head but different hats

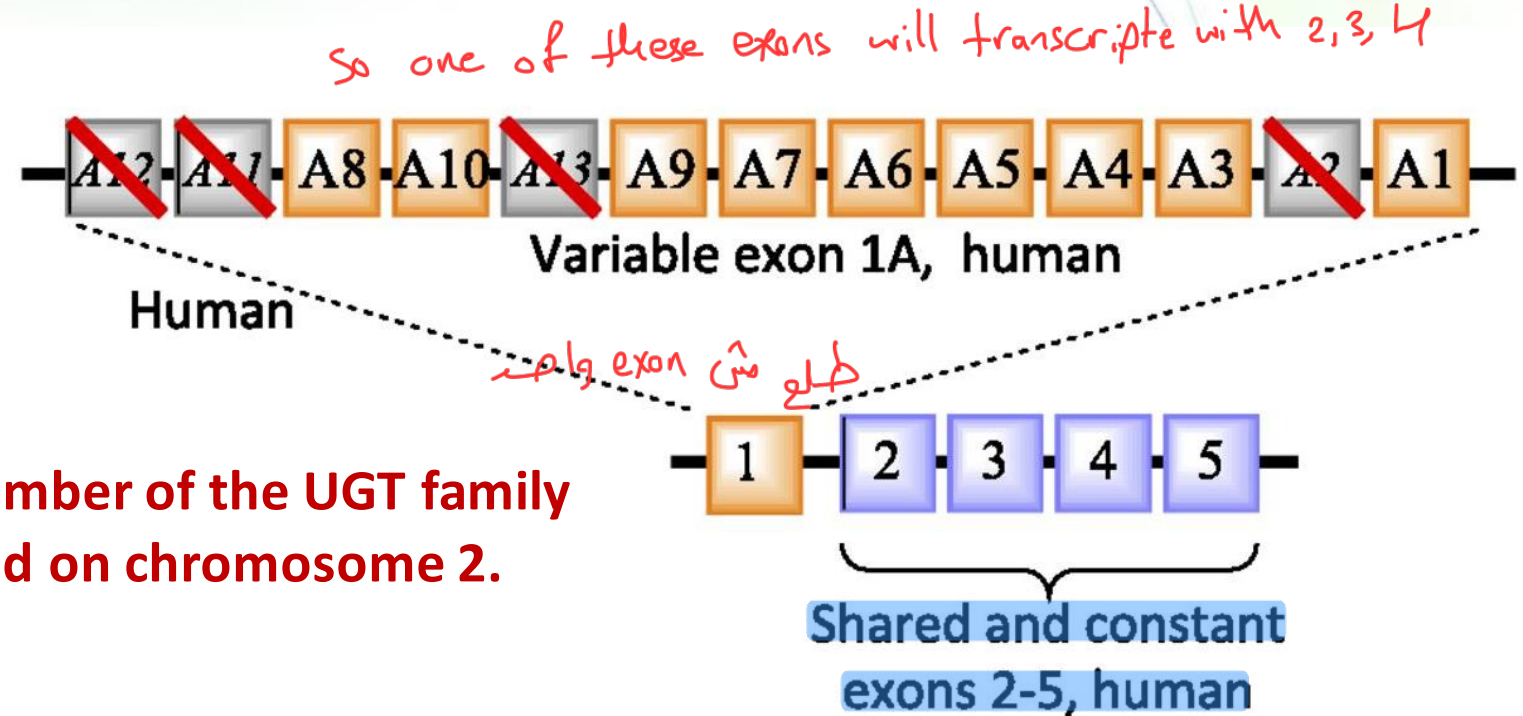
(إذا هذا نفس الراس أنا بدي أحوّل تخصصي لبستنة ومحاصيل 😂)

Then this...


The gene contains 5 exons
1
2
3
4
5
responsible for Substrate Binding So it different and cause diversity
they encode the part that add glucornate
So we need the same always
Catalytic subunit
Catalytic subunit
Catalytic subunit
Substrate binding subunit

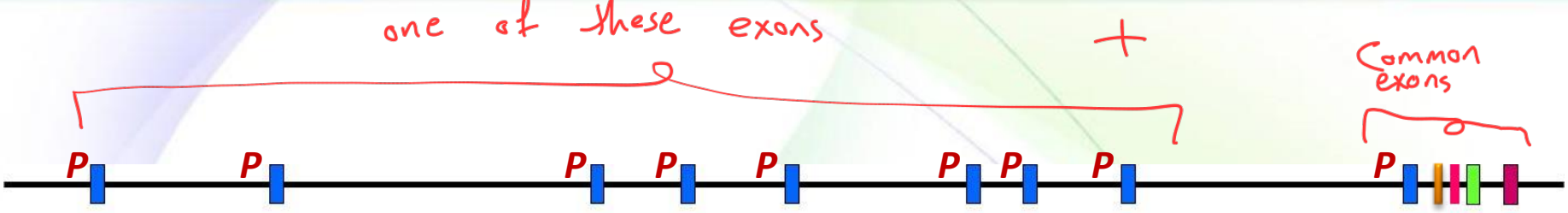
How does it do this?

- Exons 2, 3, 4, and 5 encode the catalytic domain that interacts with UDP-glucuronic acid, but…
- The 5' region of the UGT1A complex contains 9 viable tandemly arrayed first exons an, each with its own promoter.
- The 9 exons determine substrate specificity and one of them is spliced to exon 2 generating 9 possible UGT1A transcripts.

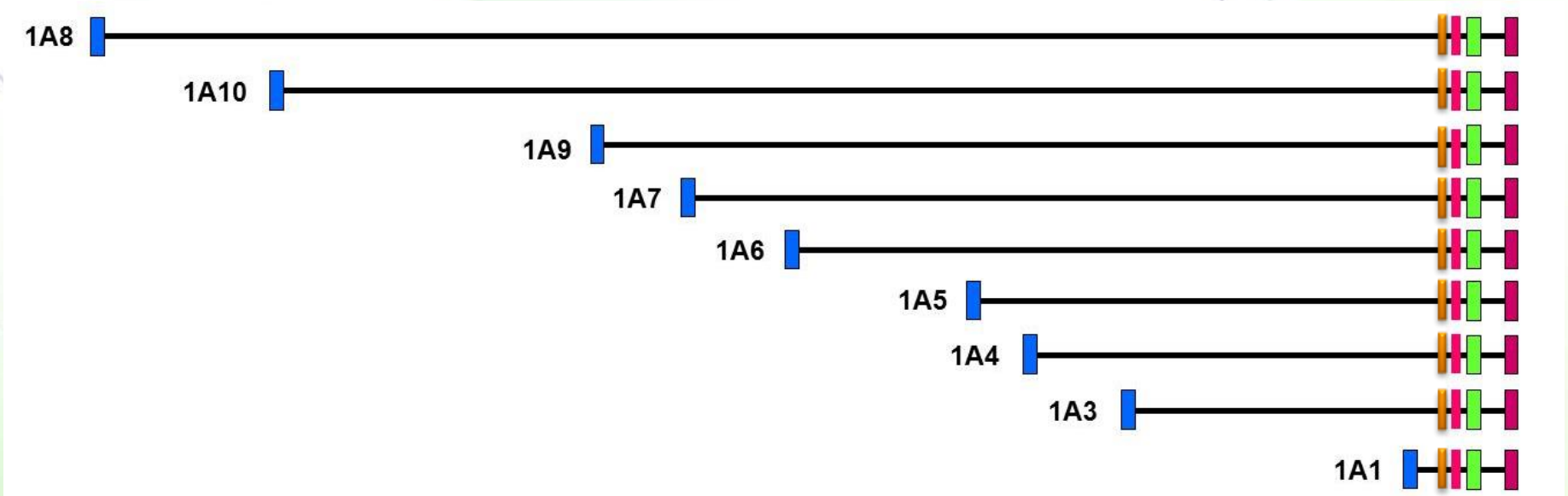


UGT1A is member of the UGT family that is located on chromosome 2.

Splice variants for UGT1A



The possible transcripts



tissue → promoter → gene → enzyme

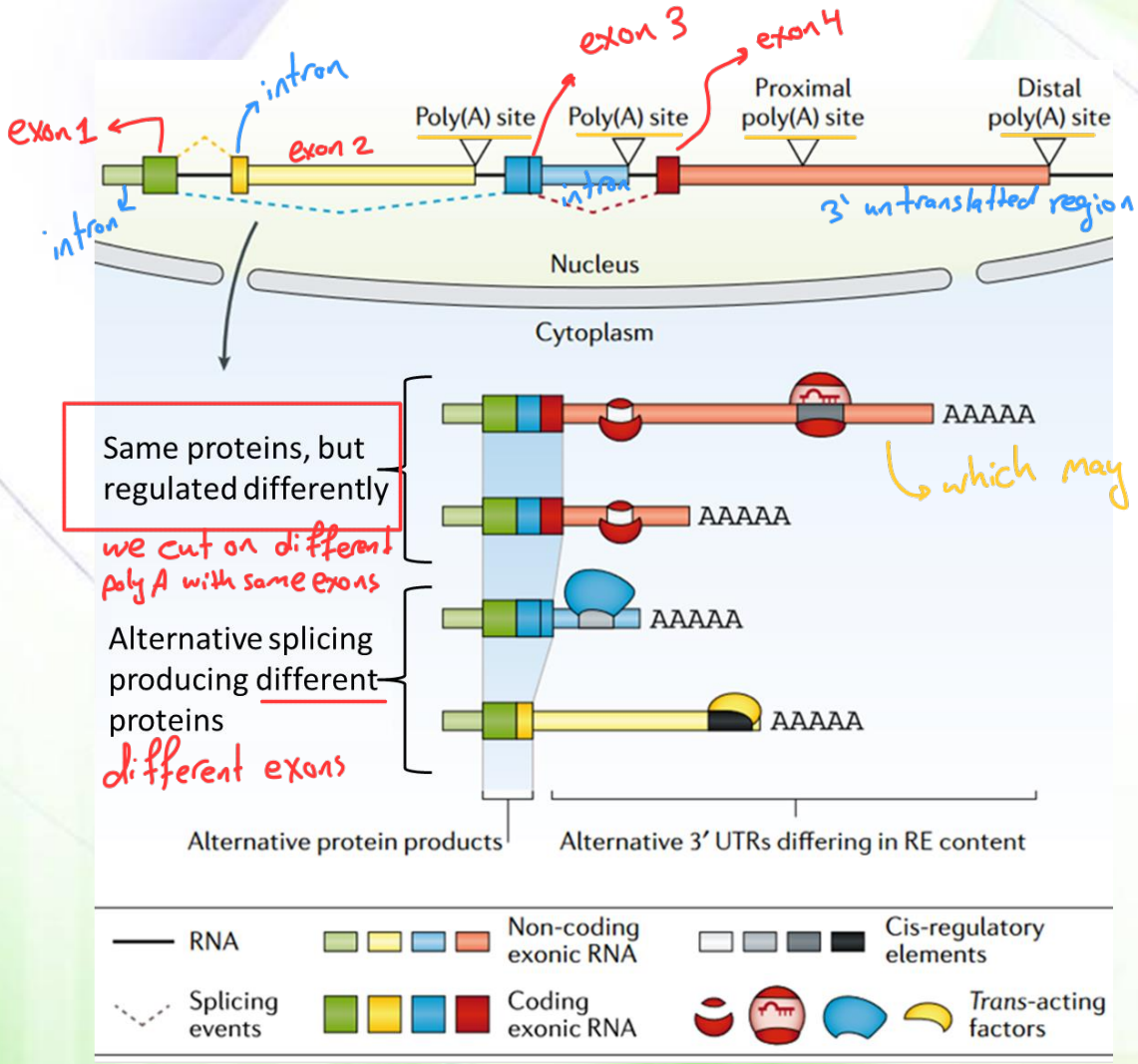
from single gene I have different genes , and the promoter determine the gene and the tissue is determine the promoter

Gene	Where expressed	Substrates
UGT1A1	Biliary tissue, colon, intestine, liver, stomach	Etoposide
UTG1A3	Biliary tissue, colon, liver, stomach	Genistein
UGT1A4	Biliary tissue, colon, intestine, liver	Tamoxifen
UGT1A6	Biliary tissue, brain, colon, kidney, larynx, liver, lung, stomach	PCBs
UGT1A7	Esophagus, intestine, kidney, larynx	heterocyclic amines
UGT1A8	Colon, esophagus, intestine, kidney, larynx	Benzo[a]phrene
UGT1A9	Breast, colon, esophagus, liver, kidney, ovary, prostate, skin, testis	Nicotine
UGT1A10	Biliary tissue, colon, esophagus, intestine, orolaryngeal tissue, stomach	Raloxifene

A third phenomenon

Alternative polyadenylation

The advantage of polyadenylation



- Changing the length of the 3'-UTR can add/remove regulatory elements.

Regulation of mRNA stability

even the RNA will not stay too long.

if maybe degredaded and the cell synthesis new one.

or we can elong RNA life to translate it.

So different level of regulation

Physiology of iron

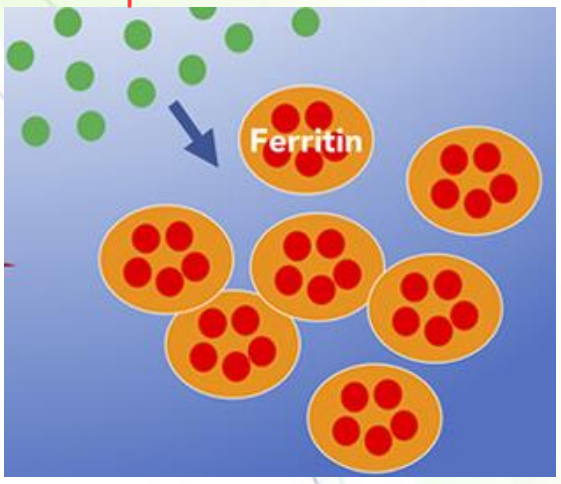
- Iron is an essential metal for the human body.
  - Oxygen transport (part of heme group which transport Oxygen in Blood
  - Enzyme function
- Too much iron can be toxic.
  - Organ failure By tissue dammage
  - Bacterial infection Because it loves iron
- The level of iron is maintained.

The players

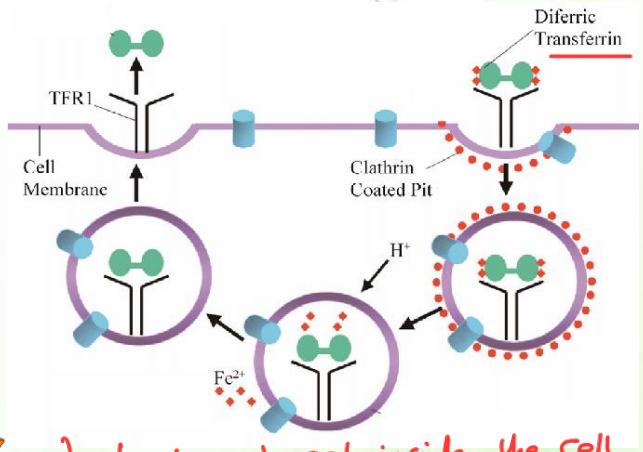
Proteins maintain iron conc. in the Body.

- Liver ferritin stores iron when abundant (in liver).
- Transferrin receptor activates iron entry in peripheral cells when needed.

So one ferritin can bind to 4k iron atoms



- When iron is high, expression of ferritin should be **up-regulated** and expression of transferrin receptor should be **down-regulated**, and vice versa.



So if the iron increase in our body the ferritin must increase to store more iron and the transferrin must be decreased to prevent more iron to move inside the cell

نفس الحكي بس ما انتبهلت

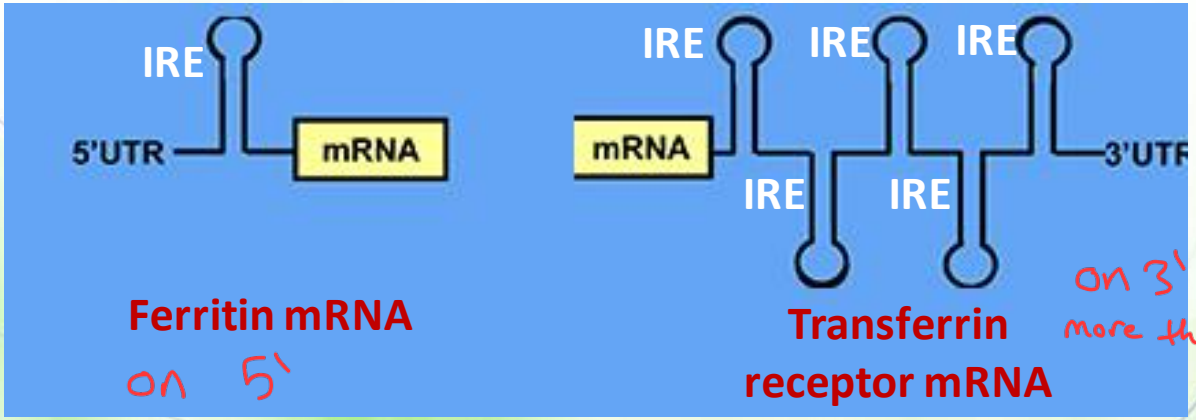
help iron to get inside the cell

* amount of receptors on the cell surface is very important

Iron-response elements (IRE)

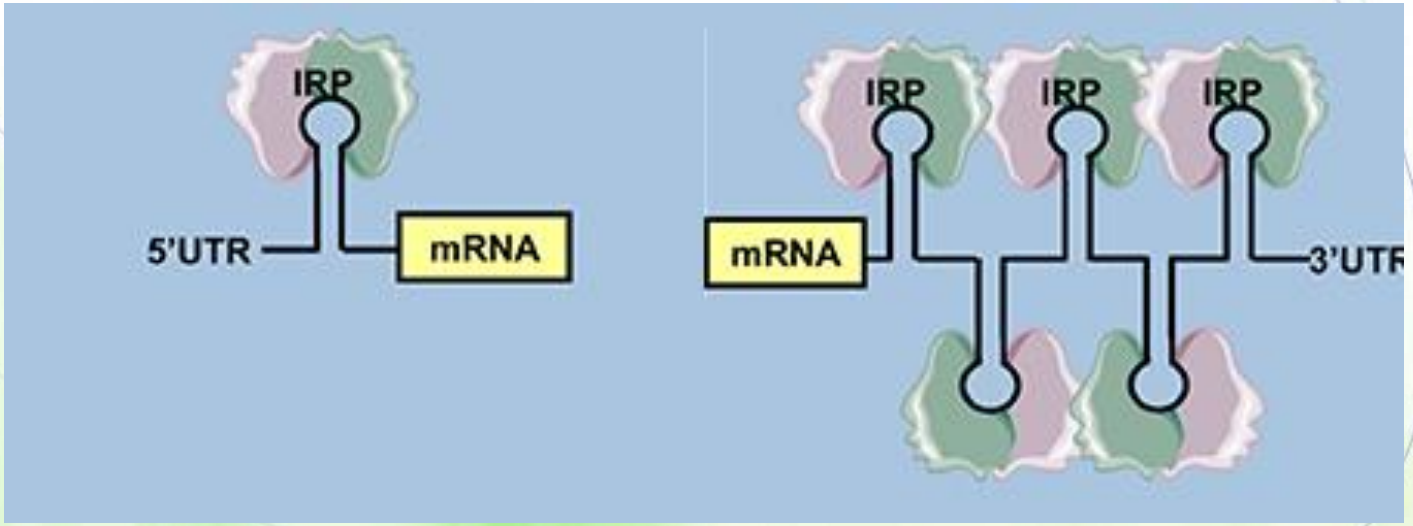
↳ RNA or DNA sequence

- In human cells, there are regions of mRNA called iron response elements (IREs).
- These regions are contained within the mRNAs of ferritin and transferrin receptor.



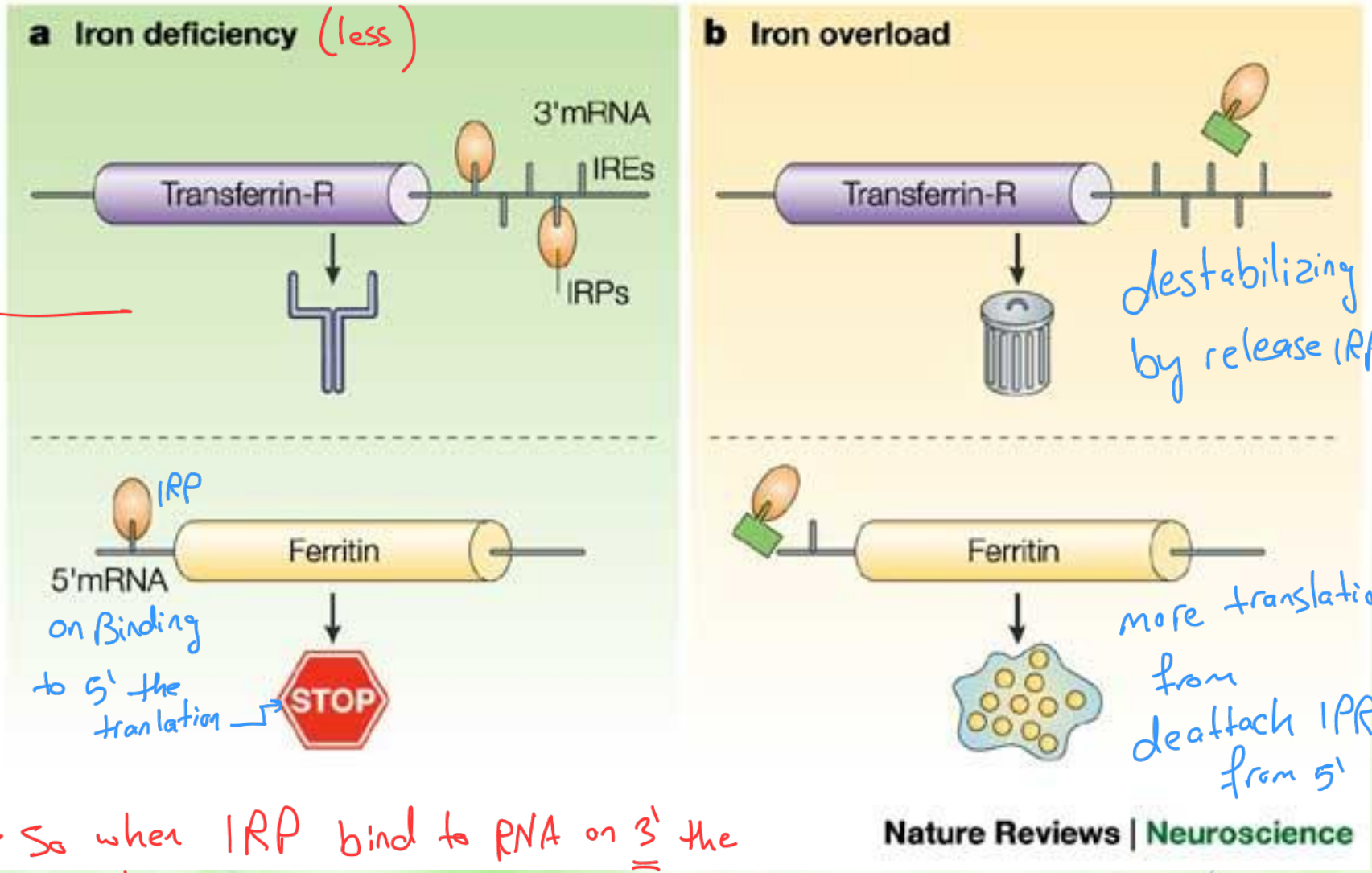
Iron regulatory protein

- Iron regulatory protein (IRP) binds to these mRNA sequences when influencing protein expression.
- However, this binding happens when iron is low.
- When iron is high, it binds to IRP preventing its binding to the IRE.



Effect on expression

- When iron is abundant in the cells, it binds to IRP, disabling the binding of IRP to the mRNAs of transferrin receptor and ferritin.
  - Transferrin receptor: mRNA is destabilized, and mRNA is degraded, lowering protein level, and, hence, iron uptake.
  - Ferritin: Translation is activated and storage increases.
- On the other hand, at low iron levels, the IRP is iron-free and can bind to the mRNAs of transferrin receptor and ferritin.
  - Transferrin receptor: mRNA is stabilized, more protein is made, and, hence, iron uptake into the cells increases.
  - Ferritin: Translation is blocked, less protein is available for storage.

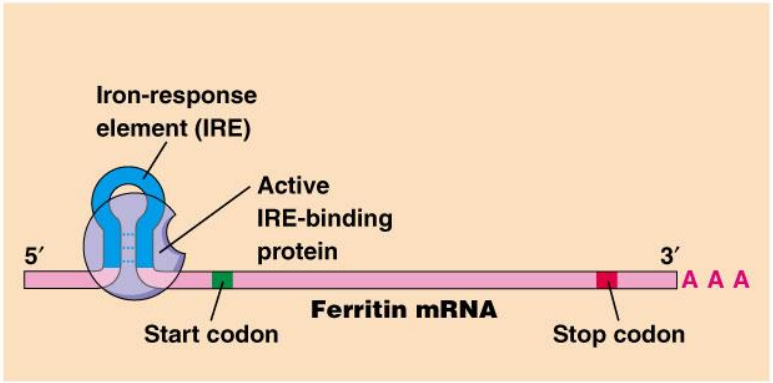


So when IRP bind to RNA on 3' the stability increase and RNA is stay longer → more translation → more protein

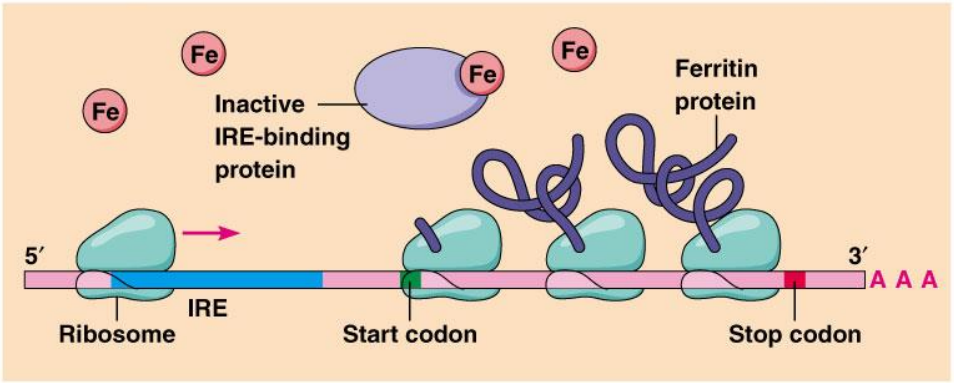
Same explination Slide

(التكرار . لعلم الشطار)

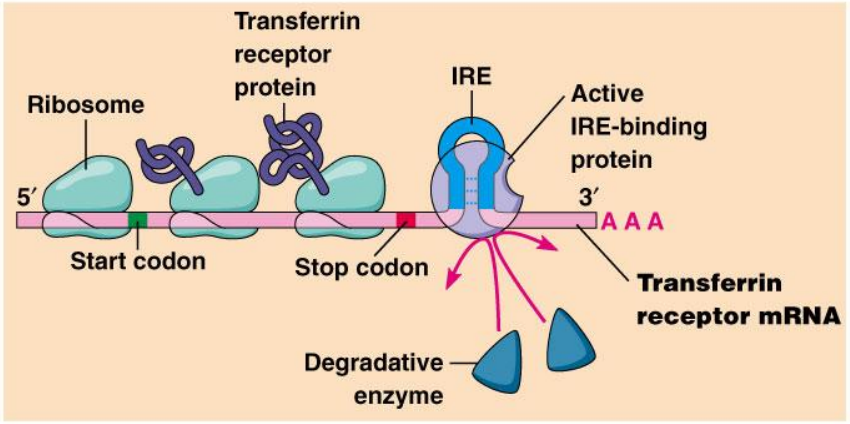
(a) Low iron concentration. IRE-binding protein binds to IRE, so translation of ferritin mRNA is inhibited.



(b) High iron concentration. IRE-binding protein cannot bind to IRE, so translation of ferritin mRNA proceeds.



(a) Low iron concentration. IRE-binding protein binds to the IRE of transferrin receptor mRNA, thereby protecting the mRNA from degradation. Synthesis of transferrin receptor therefore proceeds.



(b) High iron concentration. IRE-binding protein cannot bind to IRE, so mRNA is degraded and synthesis of transferrin receptor is thereby inhibited.