Molecular Biology (5)

Transcription

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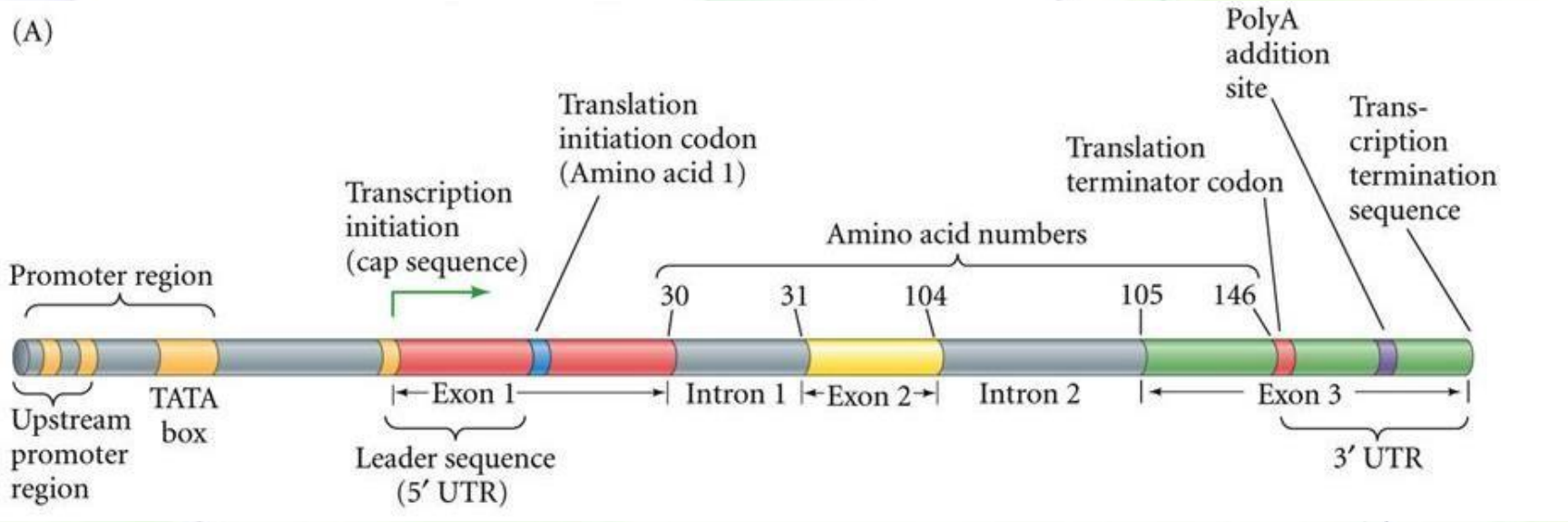
Summer semester, 2020-2021

Resources

- This lecture
- Cooper, Ch. 4, pp. 119-121, Ch. 8

Transcription in eukaryotes

Anatomy of a eukaryotic gene



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
  - 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.

Eukaryotic RNA polymerases

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

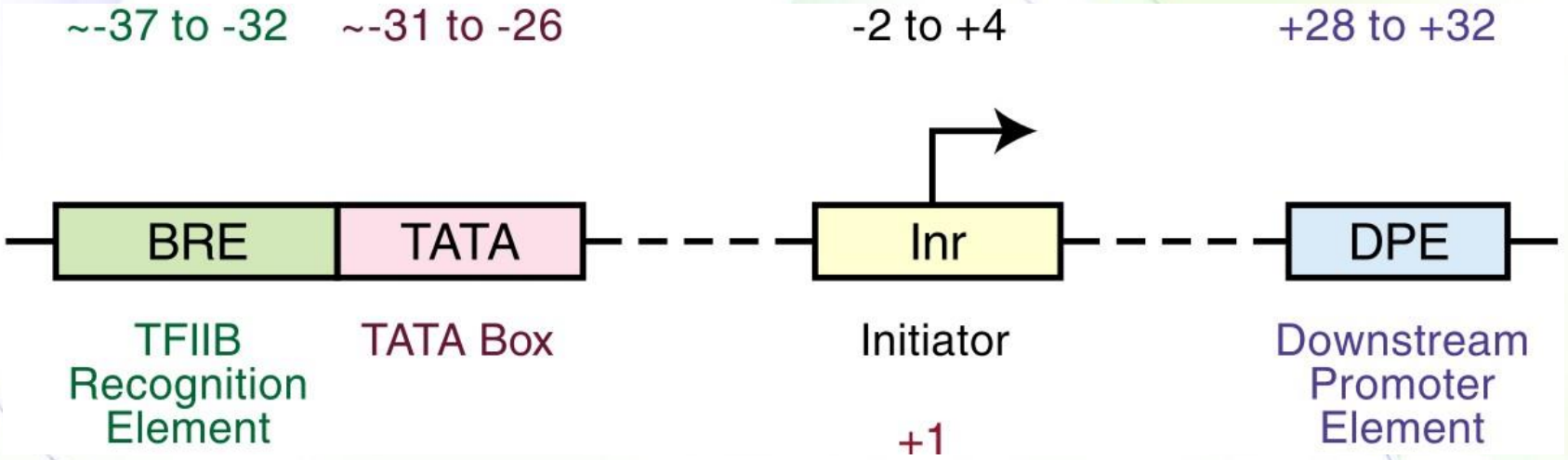
- While bacterial RNA polymerase is able to initiate transcription *without* the help of additional proteins, eukaryotic RNA polymerases cannot.
  - They require help from general transcription factors.
  - They are "general" because they assemble on all promoters used by RNA polymerase II.
  - They are designated as TFII (for transcription factor for polymerase II), and listed as TFIIA, TFIIB, and so on.

General transcription factors

- These general transcription factors
  - help position the RNA polymerase correctly at the promoter *(like what?).*
  - aid in pulling apart the two strands of DNA to allow transcription to begin *(like what?).*
  - push the RNA polymerase forward to begin transcription.

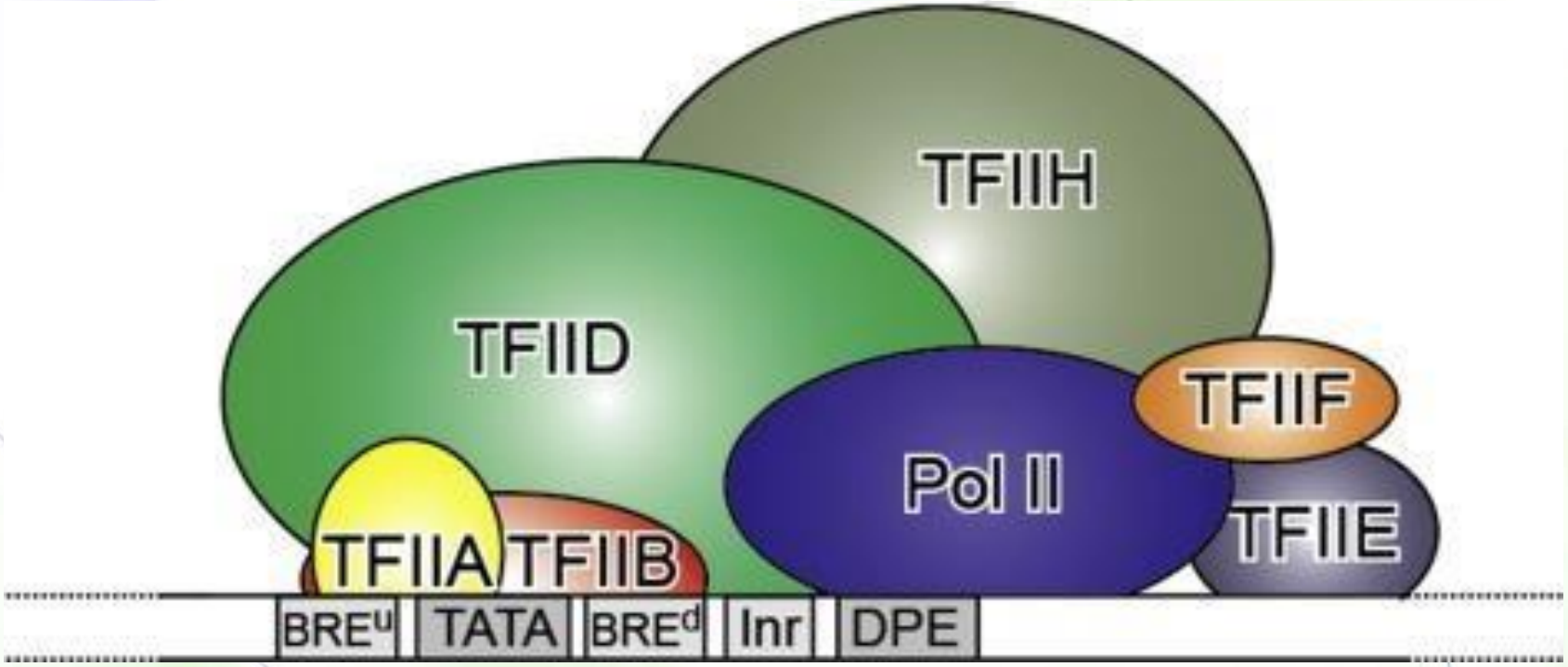
Core components of promoters

- The promoter region in eukaryotic cells is complex.



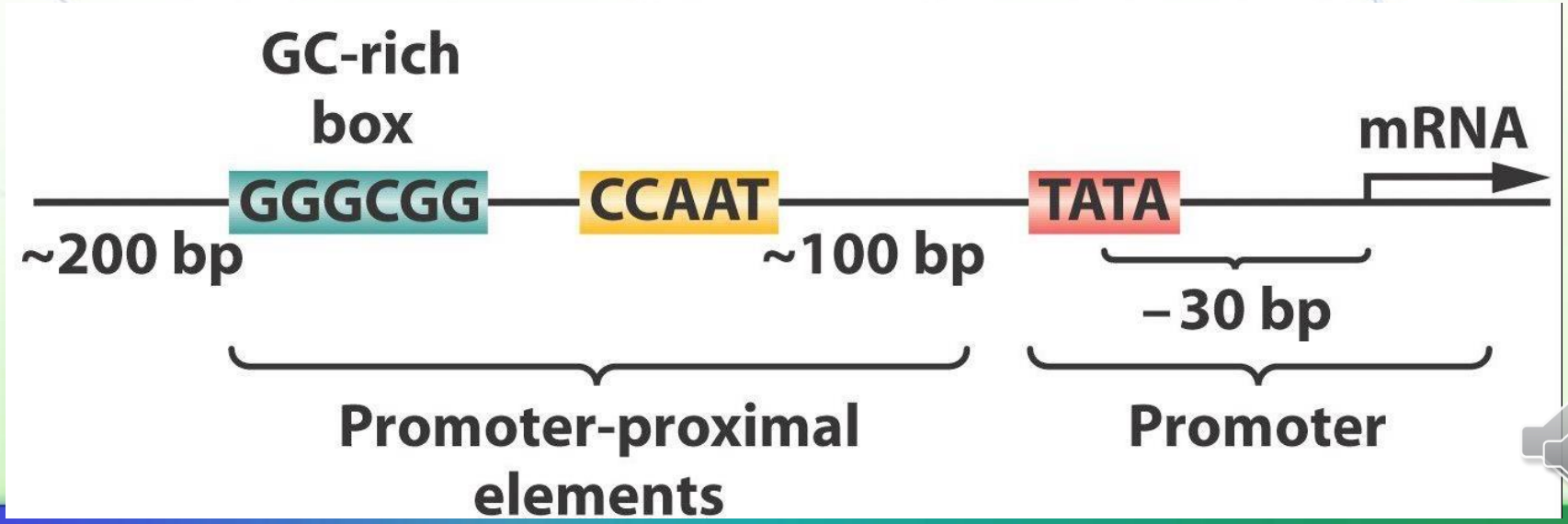
- Not all of these sequences exist at once, but genes can have a combination of these promoter elements.

Formation of preinitiation complex

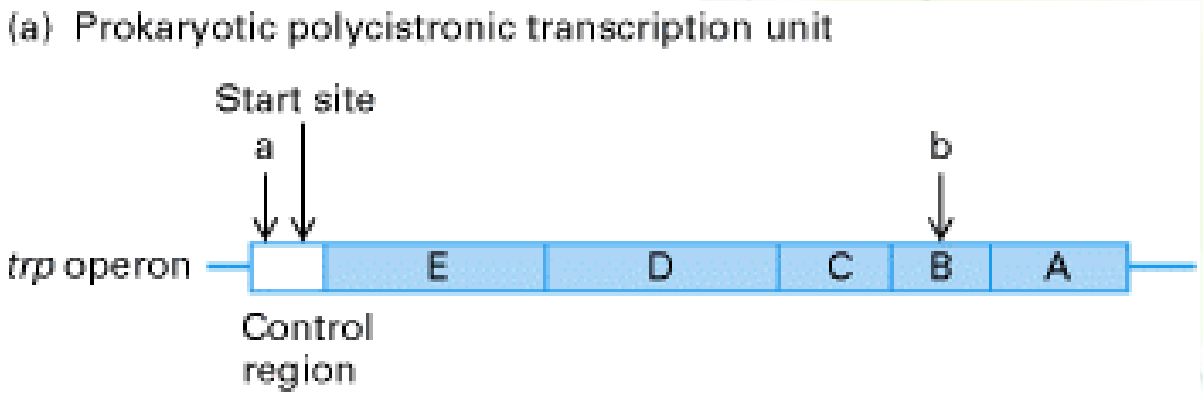


Promoter-proximal elements

- 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!

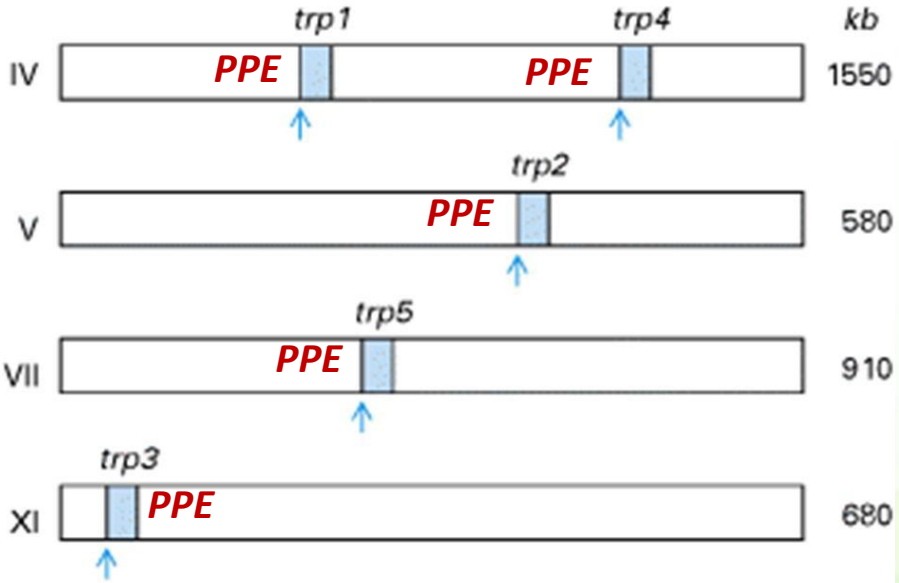


Operon vs. Proximal-promoter elements

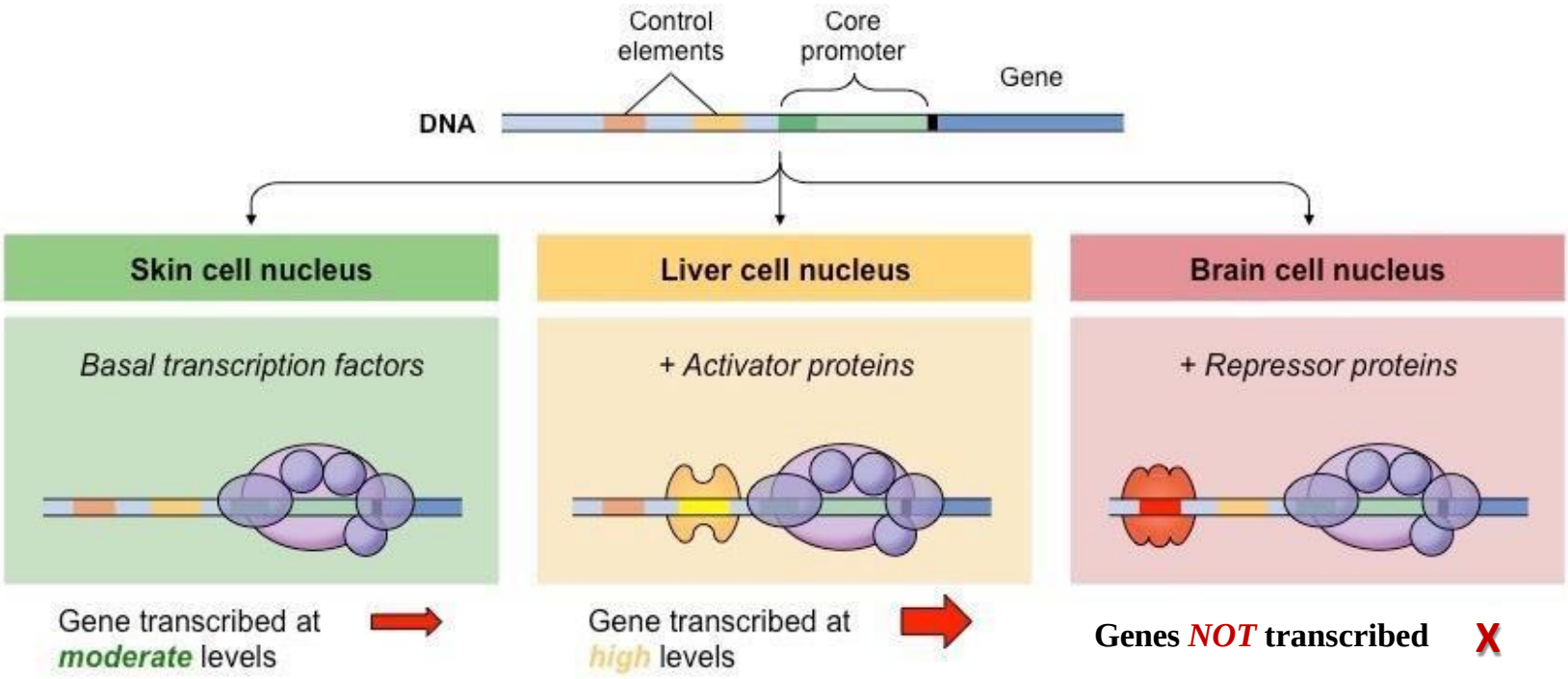


(b) Eukaryotes

Yeast chromosomes

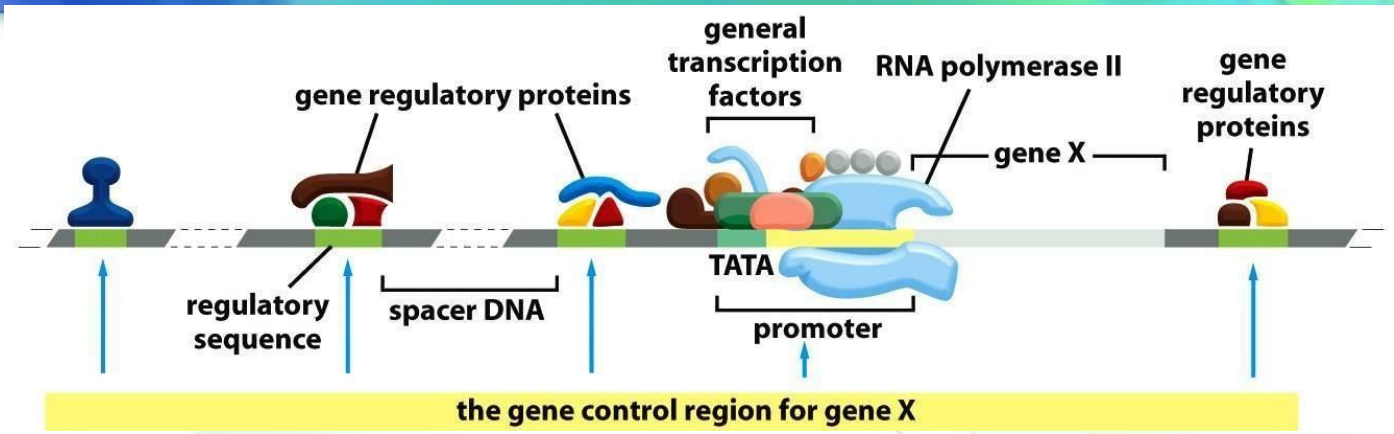


Tissue-specific transcription factors

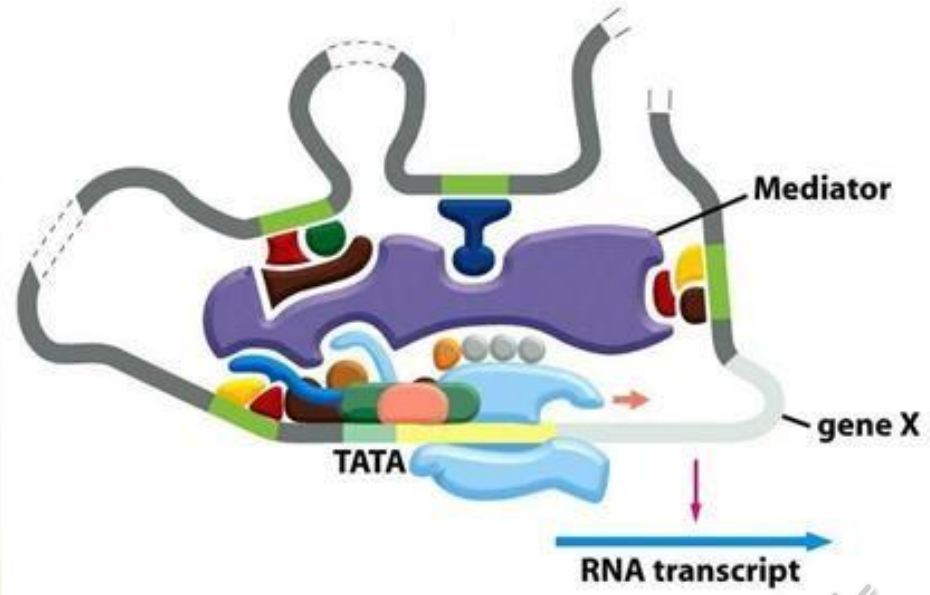


Differential expression of transcription factors (tissue-specific transcription factors) determine gene expression.

Enhancers

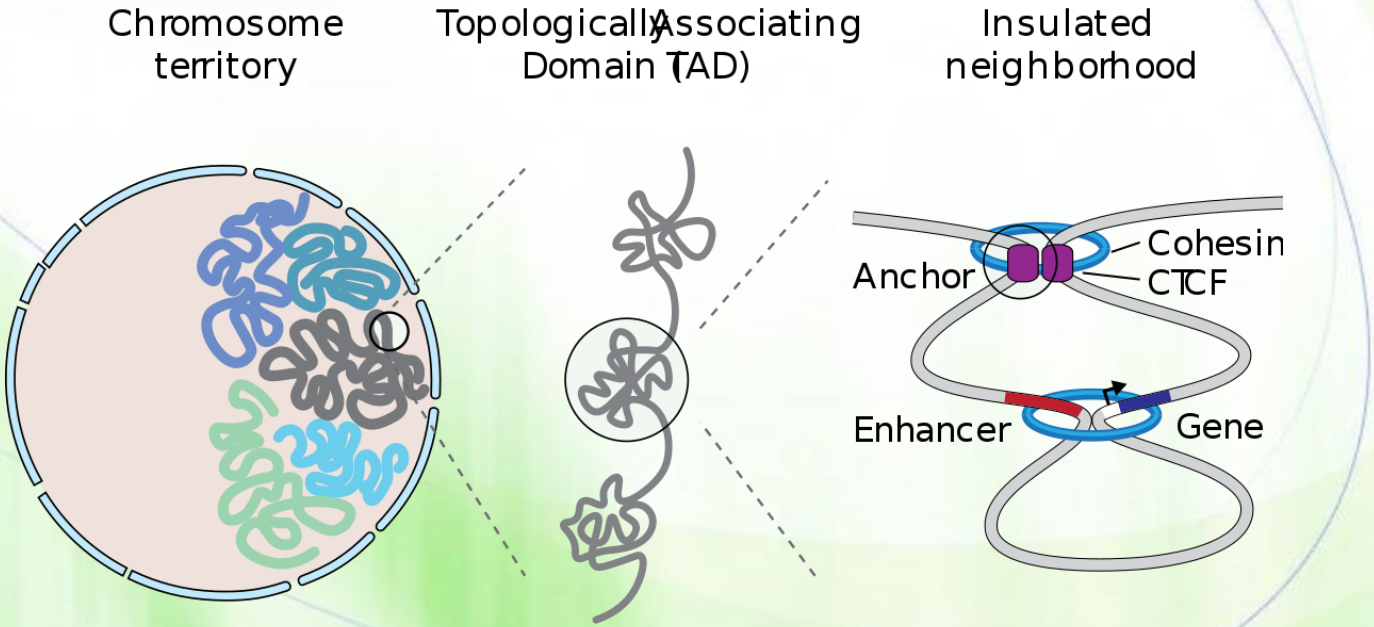


- Many genes are regulated by regulatory sequences called enhancers, which are binding sites for **specialized, gene-specific, cell-specific, regulatory** transcription factors that regulate RNA polymerase II such as a protein called the *Mediator*.
- They can regulate transcription <u>regardless</u>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

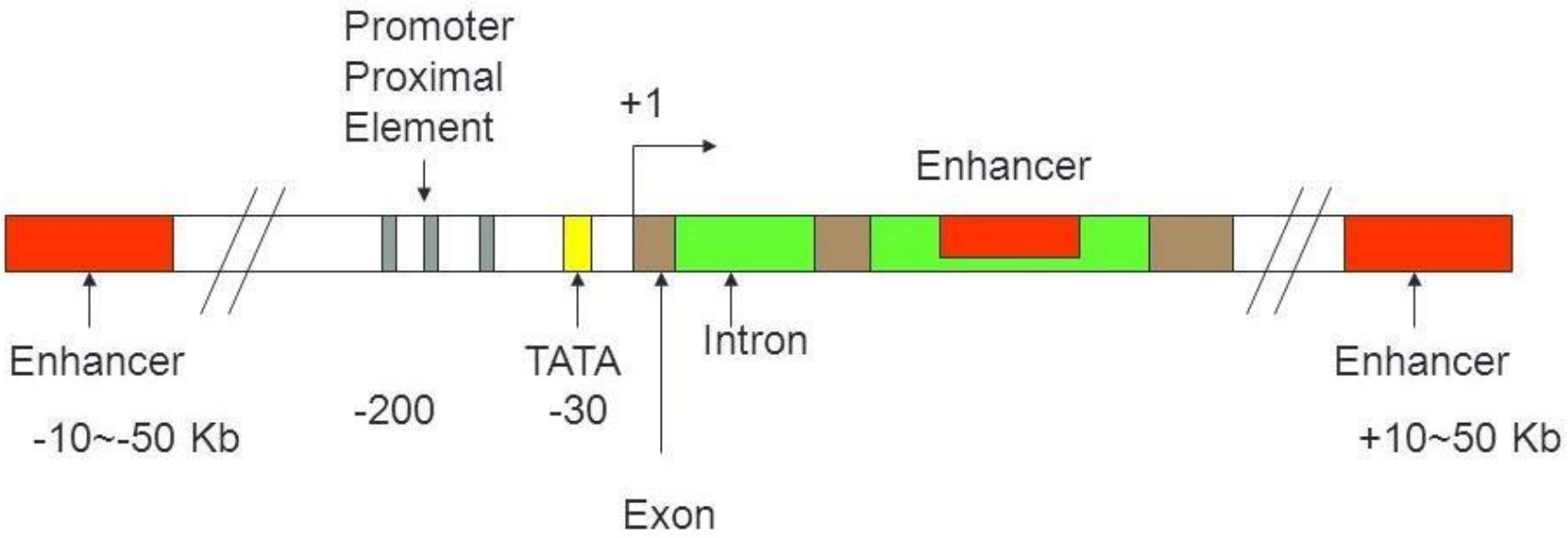


Enhancers, insulators, TADs, and CTCF

- There are 500,000 to > 1 million enhancers in the human genome (>10%).
- When loops are formed, they are stabilized by cohesion. DNA sequences known as insulators divide the genome into topologically associating domains (TADs) allowing for enhancers and promoters within TADs to interact with each other.
- CTCF proteins bind insulators and create TADs and facilitate enhancer/promoter interactions.

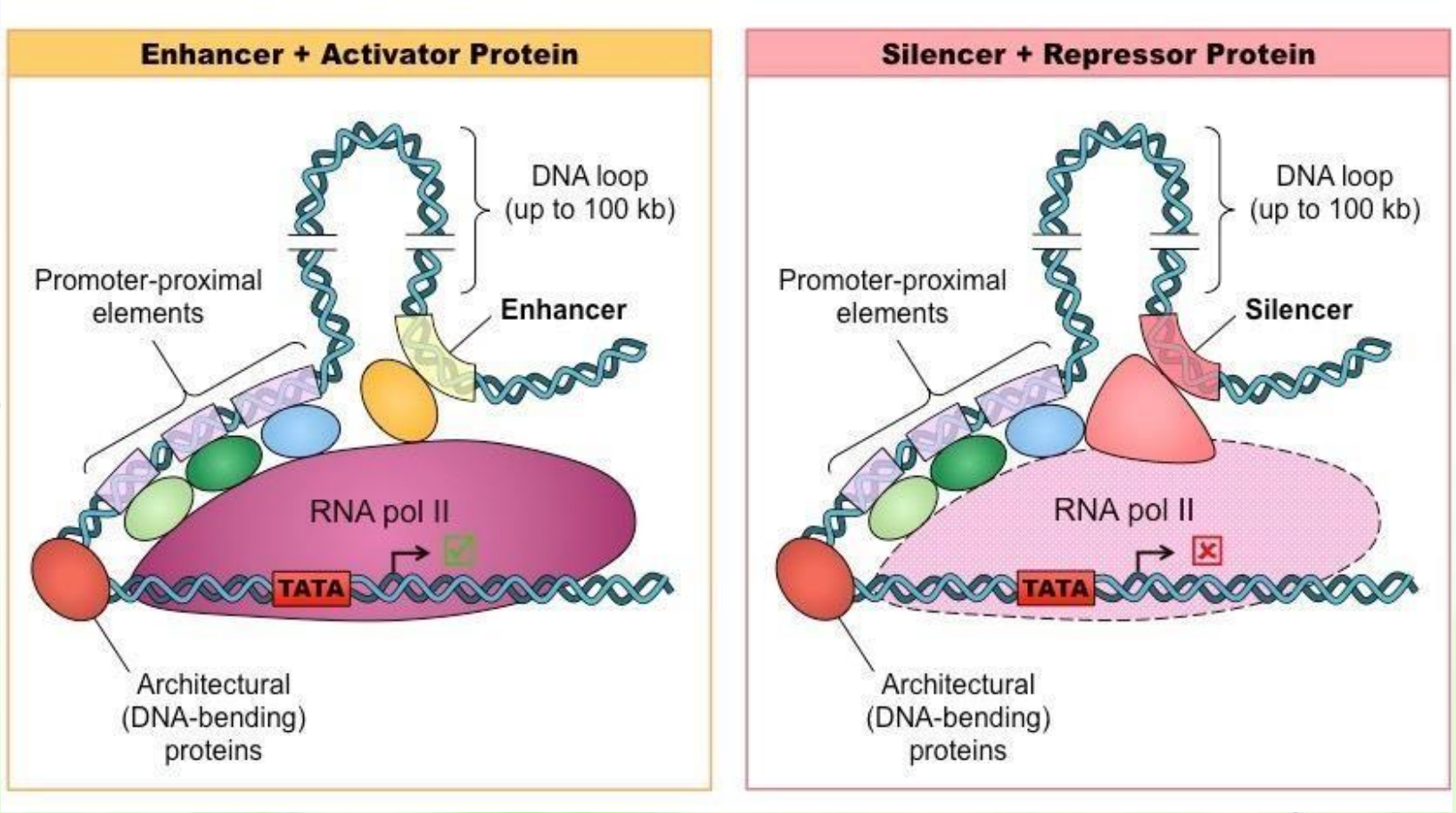


A hypothetical regulatory sequences of a mammalian gene



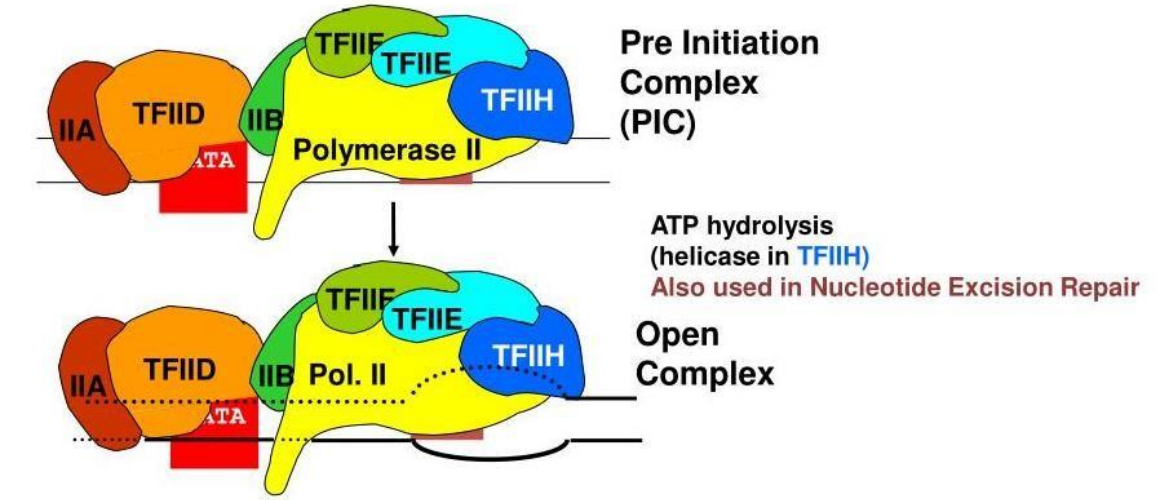
Silencers

- The opposite of enhancers.



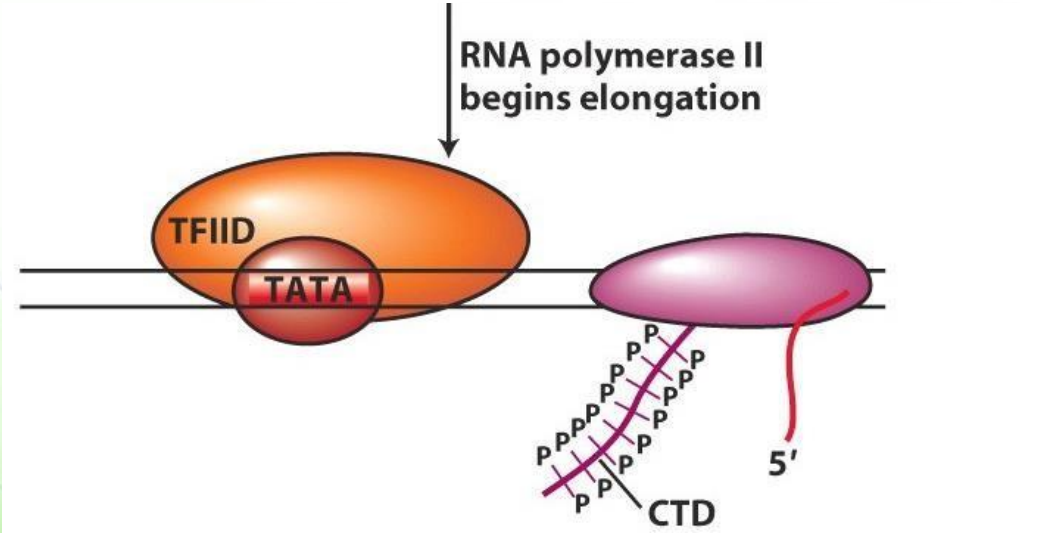
Mechanism of transcription (initiation)

- 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 polymerase.



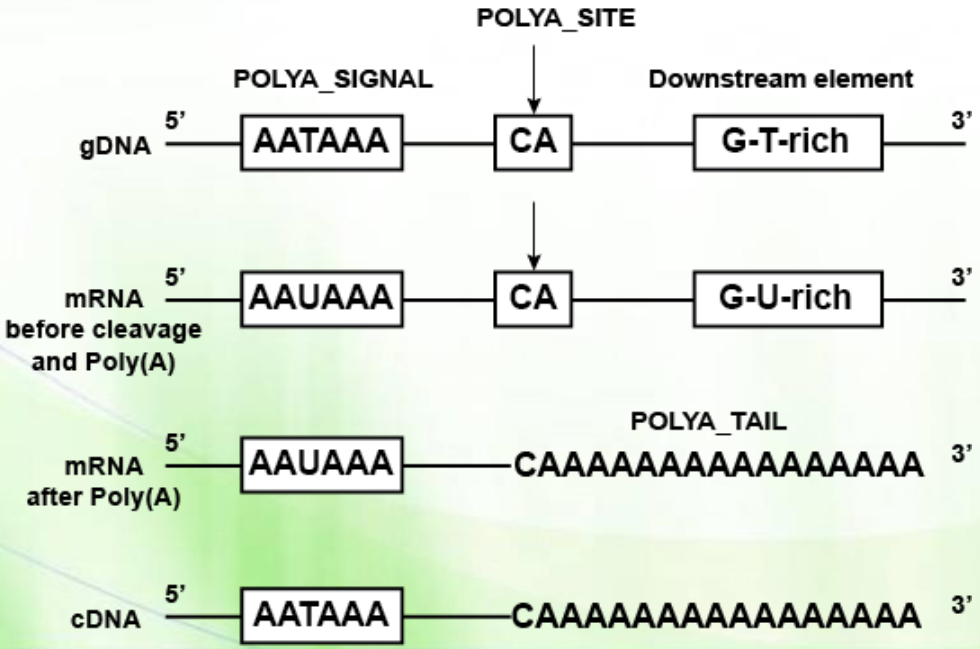
Mechanism of transcription (elongation)

- 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.



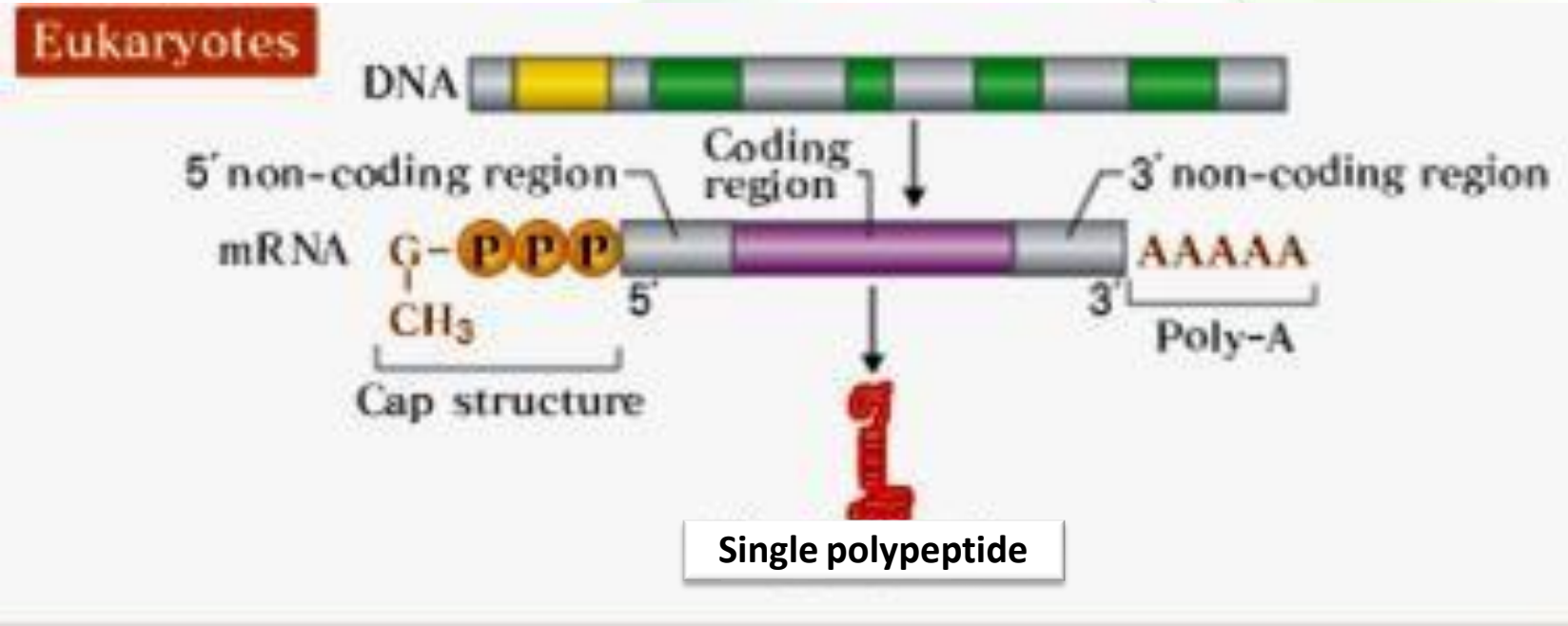
Mechanism of transcription (termination)

- 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.



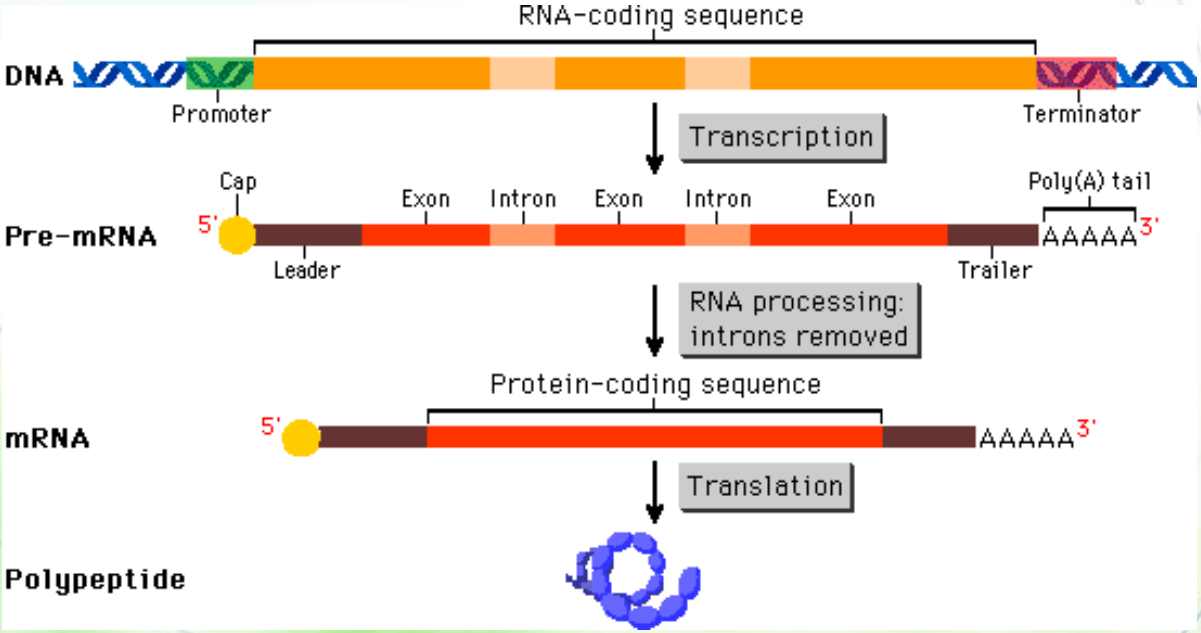
Eukaryotic genes

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



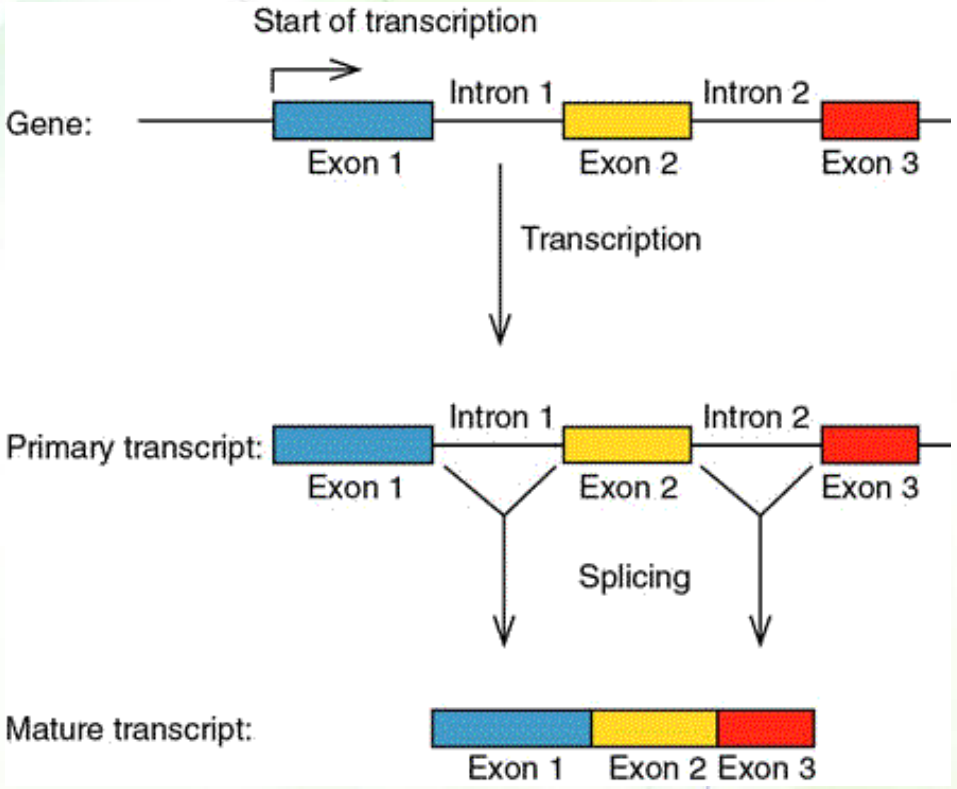
Introns vs. exons

- 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.



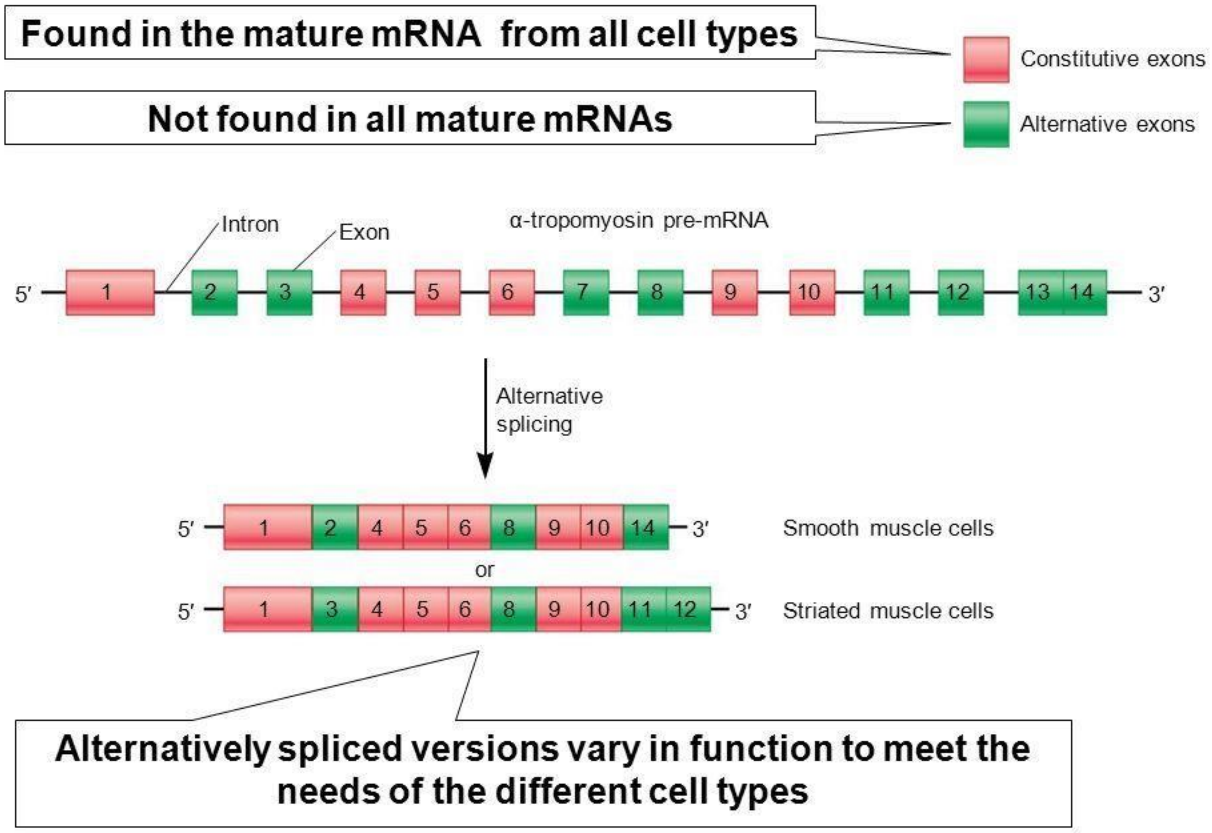
RNA splicing

- 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).



Alternative splicing

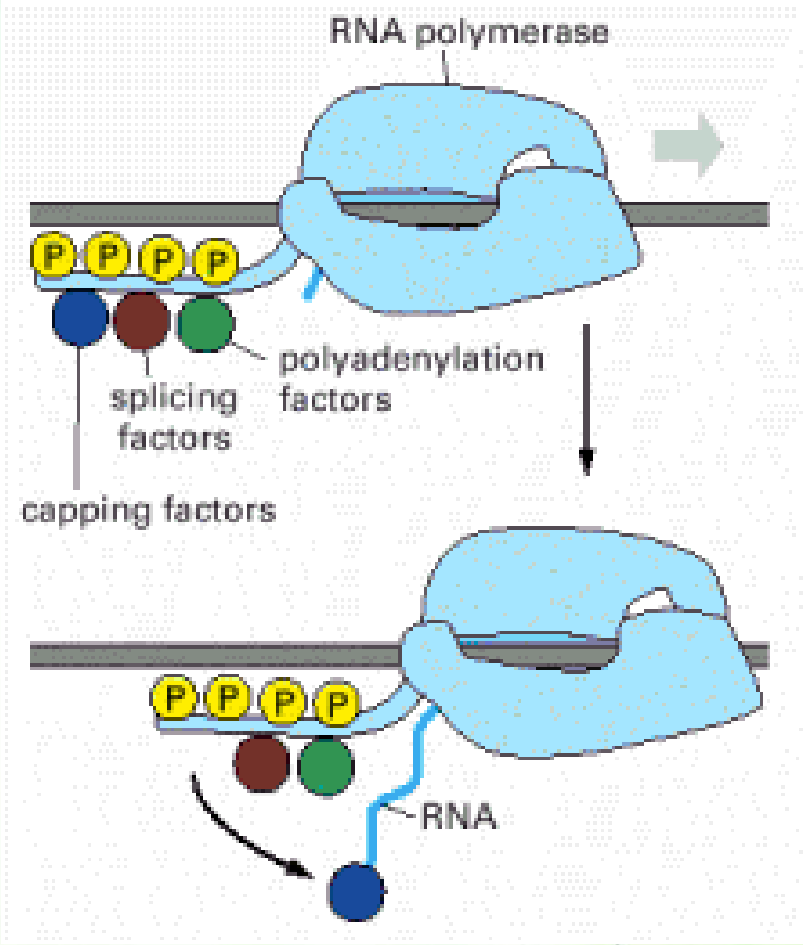
- The transcripts are spliced in different ways to produce different mRNAs and different proteins (known as protein isoforms, which are highly related gene products that perform essentially the same biological function).



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

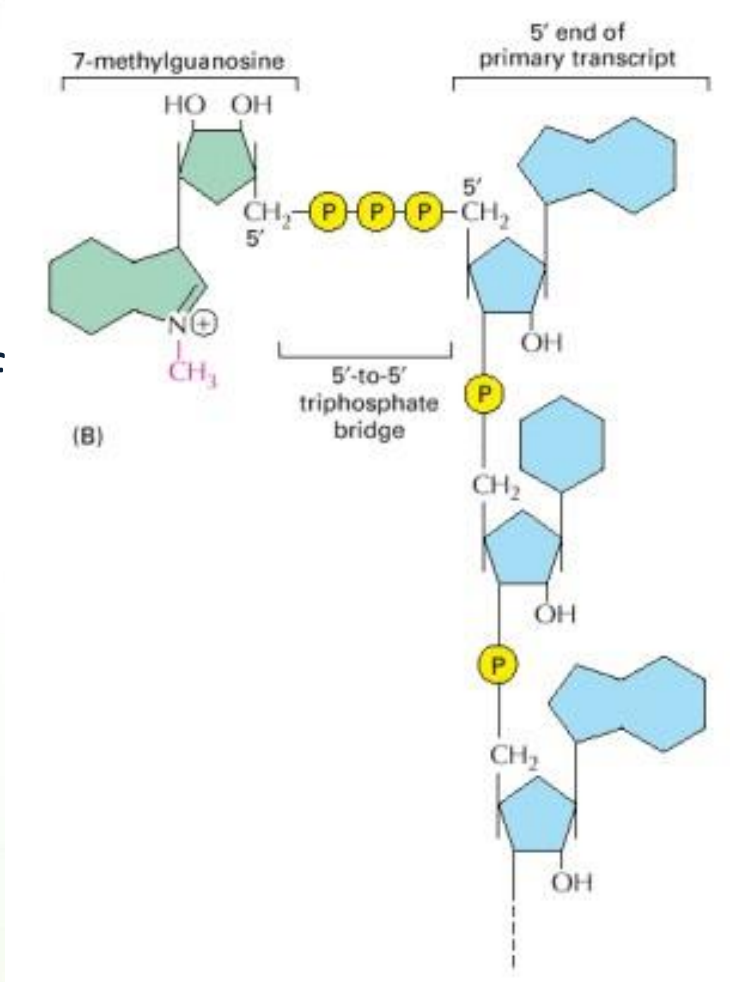
Processing of mRNA in eukaryotes

- mRNA is processed and modified extensively
  - Capping
  - Splicing
  - Polyadenylation
- 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.



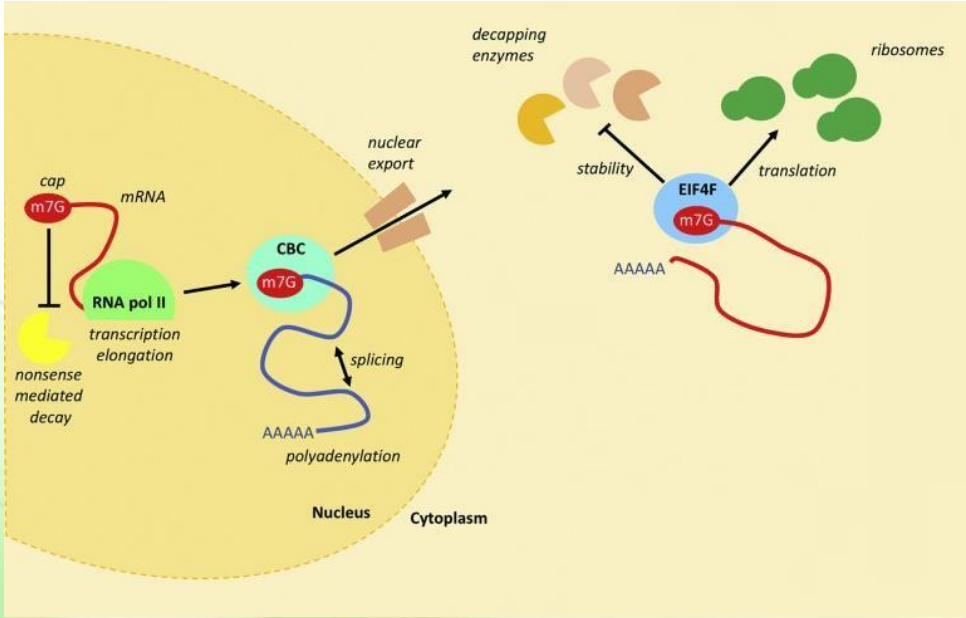
Addition of a cap

- 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’.



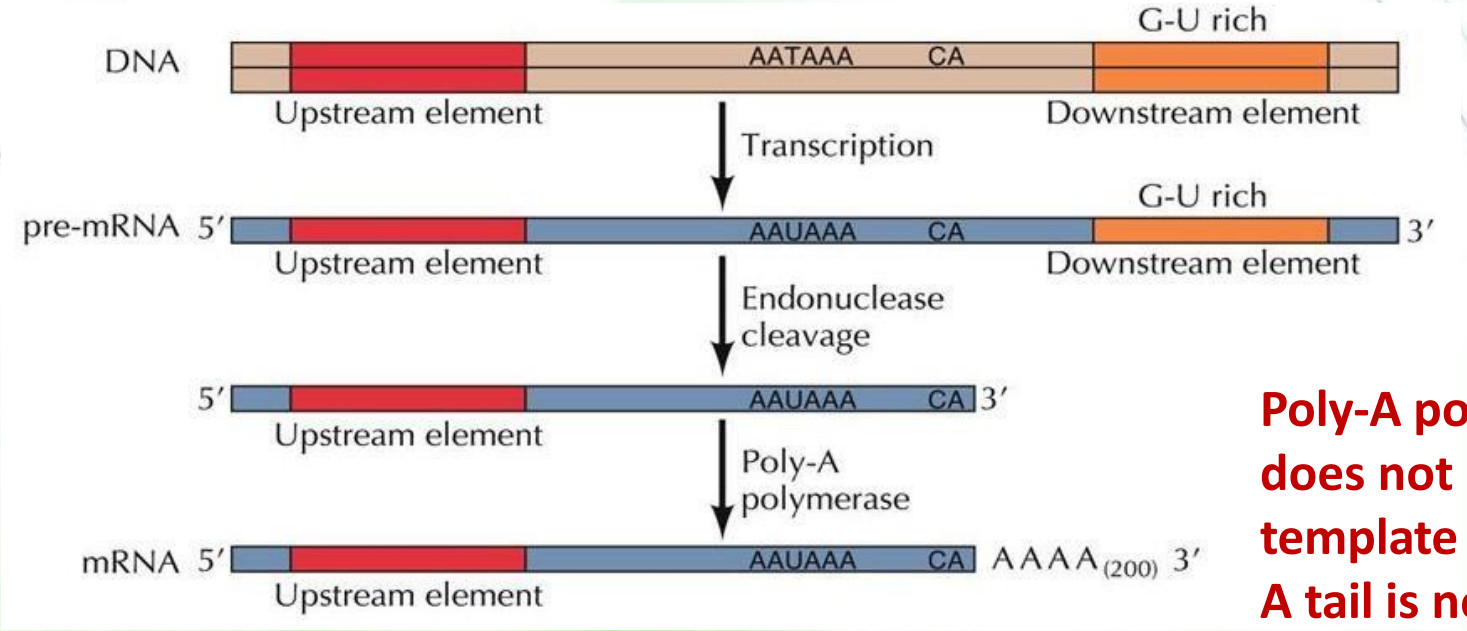
Importance of capping

- 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.



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.

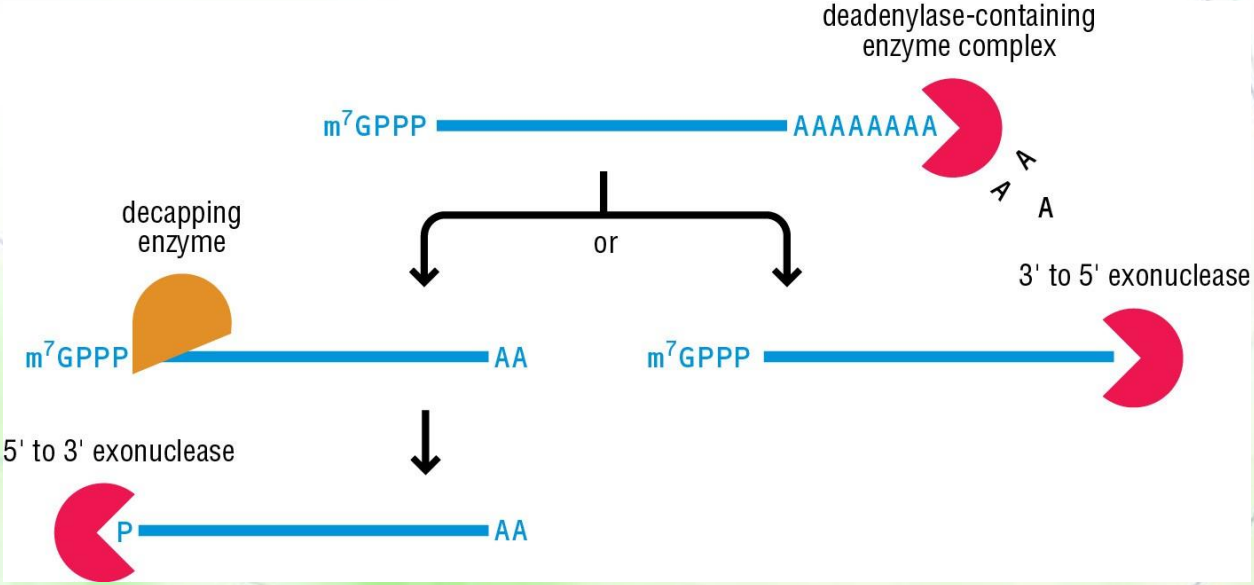
mRNA transport

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

Degradation of mRNAs

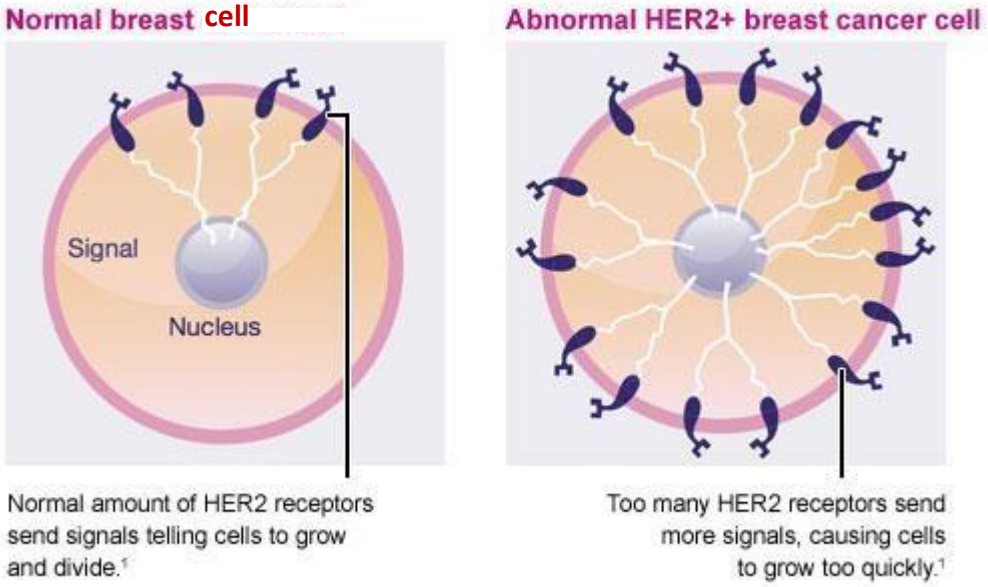
- The vast majority of mRNAs in a bacterial cell are very unstable, having a half-life of about 3 minutes.
- The mRNAs in eukaryotic cells are more stable (up to 10 hours; average of 30 minutes).
- Degradation of eukaryotic mRNA is ainitiated by shortening of poly-A tail followed by action of 3’-to-5’ exonucleases or decapping (removal of cap) and then 5’-to-3’ exonucleases .

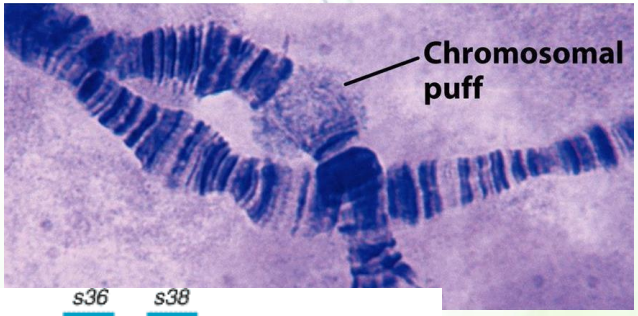


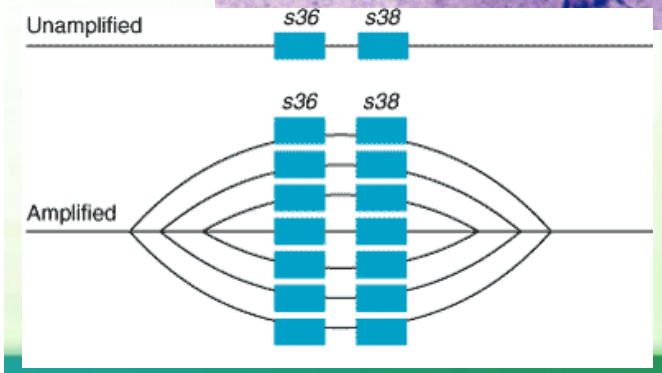
A phenomenon in eukaryotes

Gene amplification

- 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 escape resistance from methotrexate whereby the target gene, dihydrofolate reductase, is amplified.
- 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.







Regulation of mRNA stability

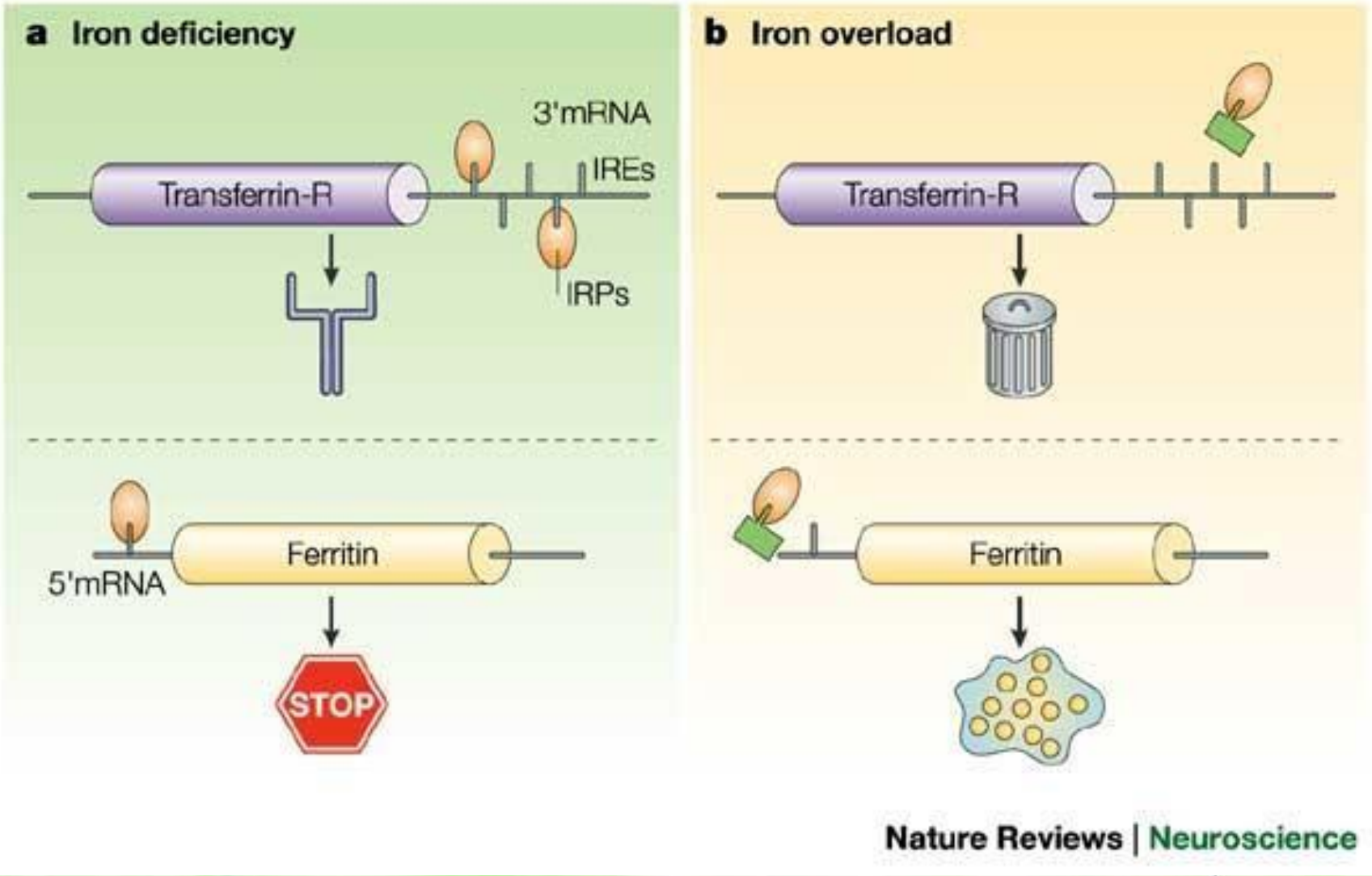
Iron-responsive elements

- In human cells, there are regions of mRNA called iron responsive elements (IREs).
- These regions are contained within the mRNA sequences that code for certain proteins that regulate the levels of iron.
  - Ferritin, transferrin receptor, ferroportin, and DMT1
- Iron-responsive element binding protein (IRE-BP) binds to these mRNA sequences influencing protein expression.

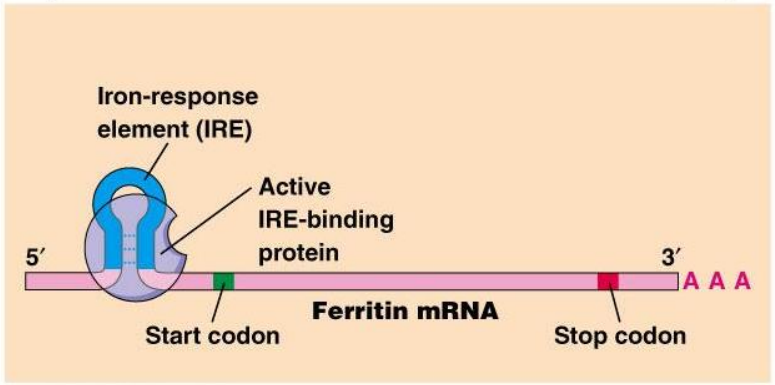
Note:
Liver ferritin stores iron when abundant (in liver)
Transferrin receptor activates iron entry in peripheral cells when needed

Effect on expression

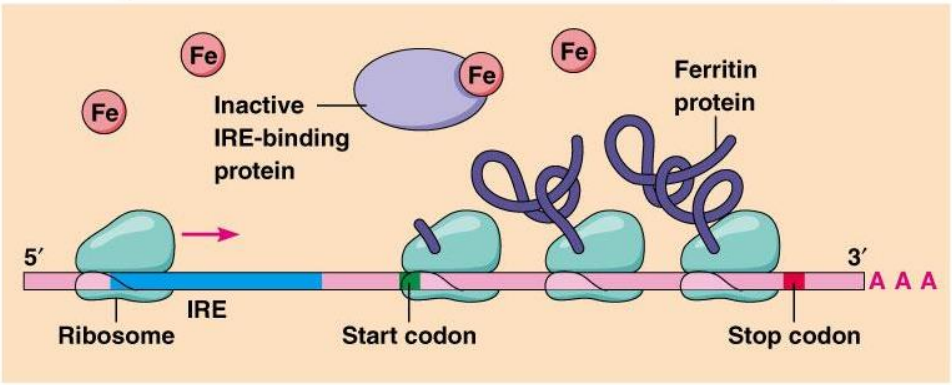
- When iron is abundant, it binds to IRE-BP, disabling the binding of IR-BP to ferritin mRNA
  - This prevents the degradation of the mRNA molecules allowing the production of more ferritin protein
  - Therefore, the iron itself causes the cell to produce more iron storage molecules
- On the other hand, at low iron levels, the IRE-BP will bind to the ferritin mRNA and, thus, the mRNA will be destabilized, making less ferritin protein
- An opposite effect is seen on the stability of transferrin receptor mRNA, which has IRE at the 3’-end.


a Iron deficiency
3'mRNA
IREs
Transferrin-R
IRPs
5'mRNA
Ferritin
STOP
b Iron overload
Transferrin-R
Ferritin
Nature Reviews | Neuroscience

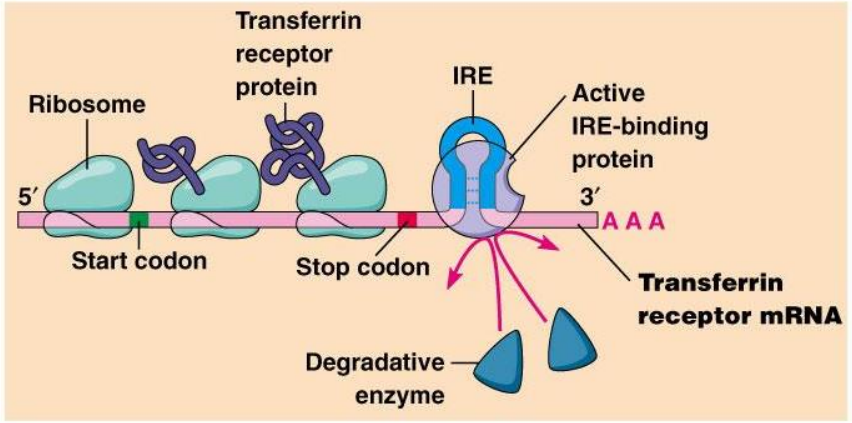
(a) Low iron concentration. IRE-binding protein binds to IRE, so translation of ferritin mRNA is inhibited.



(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.