CYTOLOGY

Premed 2018 - JU

◉ Sheet ○ Slides

Number

15

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"In order to succeed, we must first believe that we can. "–Nikos Kazantzakis.

As we keep receiving determination of our educators, with their glory of success flowing between our dreams, we congratulate Dr.Mamoun Ahram & his team of students for receiving award for their research project as they published a study visualizing a micro DNA in one type of brain tumors in the medicine school scientific day 11-April-2019 and moving to stage two with it.

We have about 21000 genes in our genome, but typically we have about 100000 protein needed. so how could a lower number of genes encode for such a large number of proteins?

-one of the mechanisms is ***alternative splicing.***

As we know the gene is composed of a group of exons& introns.

The alternative splicing is a process in which the same gene will give similar protein but in a different shape each time (protein isoform) because of the different exons expressed each time.

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).

Ex: Tropomyosin (actin binding protein, needed by all types of cells) has a function in the smooth muscle cells that differ from its function in striated muscle cells, results show that the Tropomyosin in smooth muscles needs specific exons (1+2+4 for example), while Tropomyosin in striated muscles needs other exons (1+3+4 for example), so this mRNA produces protein isoforms that can function differently to meet the need of different cell types.

-The constitutive exons have to always appear in the mature mRNA, while the alternative exons don't have to.

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

Remember: there is no single independent protein that can function by its own, it's all about interacting with other proteins and molecules, thus we can say that the protein functions by modification.

Processing of mRNA in eukaryotes

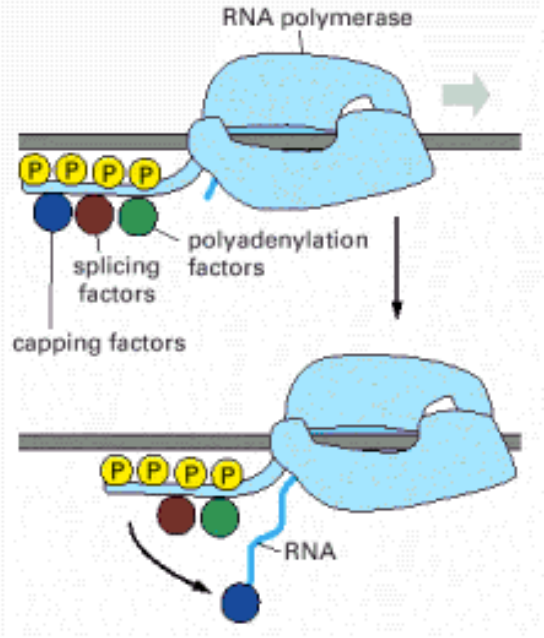
The RNA polymerase interacts with other proteins forming pre-initiation complex. A Preinitiation complex is made of RNA polymerase ll+ the general transcription factors (2a, 2b, 2d, 2h ….), the complex waits for a signal that makes it move forward synthesizing the RNA.

The signal is done by a protein that has a kinase activity → TF2H.

TF2H phosphorylates the tail of the RNA polymerase ll, thus the RNA polymerase ll can move forward doing its job (transcription).

Another process which accompanies the transcription is when the negatively charged phosphate groups on the tail of RNA polymerase allow it to interact with other specific proteins → (the capping factors / splicing factors / polyadenylation factors).

These different proteins jump on the mRNA modifying & changing it.



The m RNA is processed extensively by three processes:

1-Capping.

Once the RNA polymerase has synthesized about 25 mRNA nucleotide in a short time after its phosphorylation, a modification can now take place.

The capping factors jump on the mRNA adding a cap to determine the orientation (the beginning of the mRNA which is the 5' end).

The cap = An inverted methylguanosine ribonucleoside (GTP in reverse orientation) bound by a covalent bond.

The addition of a cap goes in the 5' → 5' direction not 5'→3'.

The importance of capping is:

a-Marking the beginning of the mRNA: This helps the cell to distinguish mRNAs from the other types of RNA molecules, which are uncapped, so the cap binding proteins (complex) CBC can bind to the cap & transport the mRNA out of the nucleus to the cytoplasm.

b-Stabilizing the mRNA by preventing its degradation that could be carried out by RNases that degrade it by breaking the phosphodiester bond & removing nucleotides.

c-The 5'-methyl cap also has an important role in the translation of mRNAs to proteins as they are a sign for the ribosomes to bind to the 5' end and start translation.

2-splicing factors.

They are proteins that jump on the tail of the RNA polymerase ll and scan the mRNA while it is being synthesized marking the beginning and the end of introns on it, the moment when the mRNA building is finished the introns are spliced (cut out).

3-Polyadenylation.

The polyadenylation factors (termination proteins) recognize an AAUAAACA (TTATTTGT on the DNA) sequence followed by a GU rich region at the 3' end of mRNA knowing that the mRNA synthesizing is done, so they cut the GU rich region terminating the transcription.

This is followed by the Poly-A polymerase (enzyme) binding to the CA region which adds 200 Adenine nucleotides. (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:

a- It help in transporting mRNA from the nucleus to the cytosol regulating it; actually scientists don't know a lot about the regulation of this process, but they know that mRNA must be exported out of the nucleus & un-exporting it means that it's defected and does not have the A tail for example. (Defective mRNA molecules could be interrupted RNA, mRNA with inaccurate splicing, and so on.).

b- It helps in translation.

c- It stabilizes mRNA. In this field, we would like to discuss the following:

when a specific protein is not needed by the cell it is metabolized and we must turn off the gene expression that produces it, so the m RNA that would be translated into that protein will get defected, it won't leave the nucleus and it undergoes a controlled degradation.

In the bacteria the mRNA is highly unstable with a half-life of about 3 minutes, in eukaryotes it is more stable and some can last for 10 hours (sometimes up to 24 h) with an average of 30 minutes, the degradation occurs by an enzyme that has a deadenylase activity (removal of adenine nucleotide) that removes the poly A tail or makes it shorter followed by exonucleases function that removes nucleotides from the 3'→ 5' direction. Another path could follow the poly A removal through the degradation by the decapping enzymes that remove the cap followed by exonucleases function.

-the resultant free nucleotides must be recycled; they undergo phosphorylation making them triphosphates rather than monophosphates.

- the 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.

Gene -amplification

During the DNA replication and other conditions cells may need more than one copy of a certain protein ex.: in the DNA replication we need a huge number of histones. The cell will deal with such conditions in a process known as gene amplification.

Every cell in our genome has multiple copies of a specific gene on different chromosomes (ex. The histone gene and rRNA gene which is needed to synthesize ribosomes. As the cell needs a lot of them, it must have a lot of rRNA genes.

Remember: we need huge amounts of proteins that are not classified as enzymes; because enzymes are efficient, so the cell needs a small amount of them to do a certain function.

How can we have many copies of a specific gene?

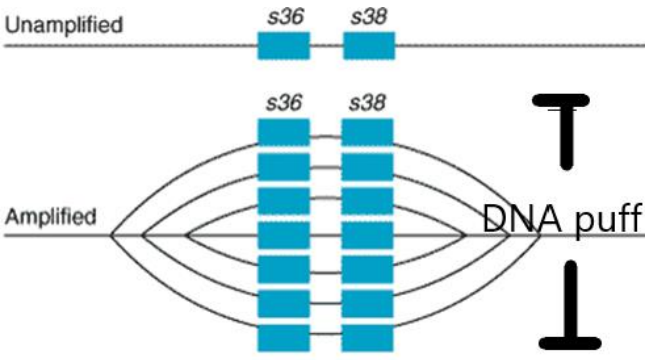
1-The cell has the same gene repeated many times on different chromosomes and they are all active.

2-*Gene duplication*: when a cell undergoes a special condition (unusual state of circumstances) it has to fight these conditions by making too many copies of a specific gene (restricted region of the DNA) on the same location of that gene on the chromosome synthesizing too many copies of that gene and thus increasing the amount of DNA in that region (DNA puff).

This is a temporary situation that happens when the cell goes through stressful condition as a way of managing; when it needs to get back to the normal state it will get rid of the extra copies.

Normally cells develop resistance against toxic materials such as methotrexate; when the cell recognizes it, it expresses the gene that would be translated into the methotrexate reductive gene (dihydrofolate reductase); but one sample of this protein is not enough; so, this gene should undergo amplification.

We expect bladder cells to be proficient in such a thing as they face many kinds of toxic materials, so they develop normal resistance by using the gene amplification.



--Nevertheless, it creates a large issue in treating cancer.

When a patient undergoes chemotherapy, we can see cancer cells developing resistance by taking advantage of a normal defending mechanism in the cell which is

gene amplification producing a lot of proteins that pump out the chemotherapeutic chemicals out of the cancer cell.

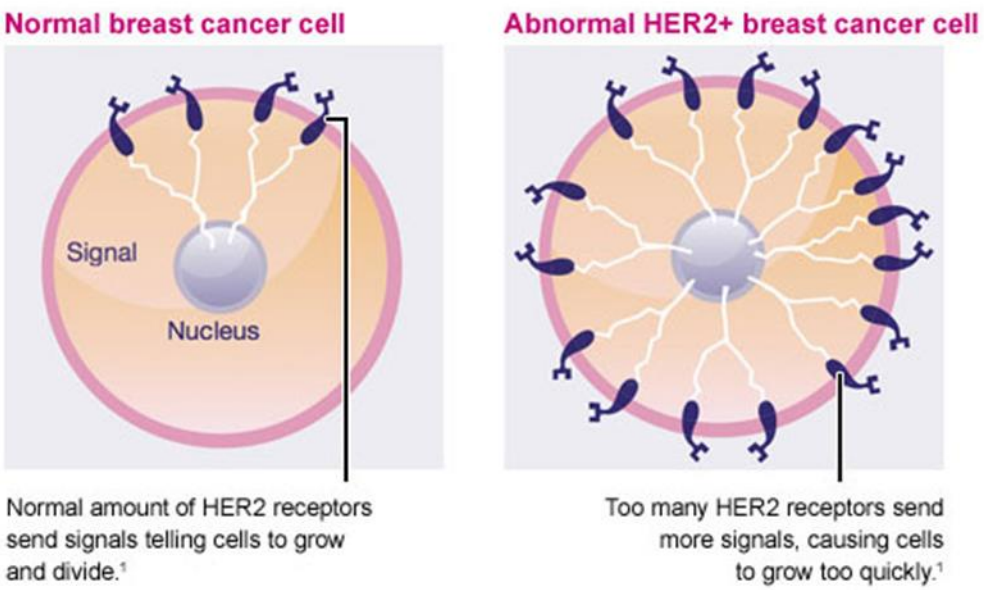
Let's take breast cancer as an example;

- 25 years ago, patients used to get a chemotherapy that kills any dividing cell, many side effects accompany this therapy such as hair loss, exhausted body, fatigue, being unable to eat, and a long list because of killing cancerous & normal dividing cells.

On the surface of the normal breast (mammary) cells, a limited number of the ''human epidermal growth factor receptor ll'' exist; which cancer cells amplify to increase deficiency of the therapy.

-Nowadays we take a biopsy of the breast tumor. If it has HER ll we give the patient a drug that targets this protein & inhibits it. Other receptors that we can do the same thing with are estrogen receptors.

If the cancerous tumor has neither HER ll nor estrogen receptors we get back to the chemotherapy. Searching for other treatments is still on.



*Iron is very precious in the body, so our bodies do not exclude it. If you picked a random person, we don't expect him to have an Iron deficiency anemia and we expect men to have higher efficiency of iron storing.

Red blood cells have a half-life of about 100 days (which is NOT too long) eventually they die => so they rupture & release hemoglobin, hemoglobin undergoes degradation releasing iron, and iron immediately binds to a protein that preserves it.

When we eat something that contains iron, iron is translocated through intestinal cells to the blood where it binds to a protein called transferrin.

We have Two possible pathways of transferrin:

a-if cells need iron:

cells that need iron will expose transferrin receptors on their surface, the transferrin that carries the iron will bind to it (and the cell is happy ☺).

b-if cells do not need iron:

then cells won't have transferrin receptors on their surfaces; transferrin will move into the liver where it binds to a protein known as ferritin.

A single Ferritin has the ability to bind to 4000 iron atoms storing them.

When cells need iron, they send a signal to the liver telling it to release iron, so iron is released from ferritin into the blood stream; cells present its transferrin receptors and iron can get into the cell.

As our bodies possess almost constant amount of iron; iron must be balanced between cells & its storage sites :-

If cells need iron→ the number of transferrin receptors ⇧ the number of ferritin ⇩

If cells don't need iron (there's an excessive amount of it)→ transferrin receptors ⇩ ferritin ⇧

So, in the body the enzymes that have opposite functions are often regulated by the same mechanism that have different effect on each one. For instance: phosphorylation can activate one protein and inhibit the other one (meaning that they are opposite proteins) in order to keep balance.

There is an element (element→referring to nucleotides sequence) exist on the mRNA of both ferritin and transferrin receptor.

This element is specific for a protein known as Iron Response Element binding protein.

Iron-responsive element binding protein (IRE-BP) binds to these mRNA sequences influencing protein expression coding for certain proteins that regulate the levels of iron like:- Ferritin, transferrin receptor, ferroportin, and DMT1.

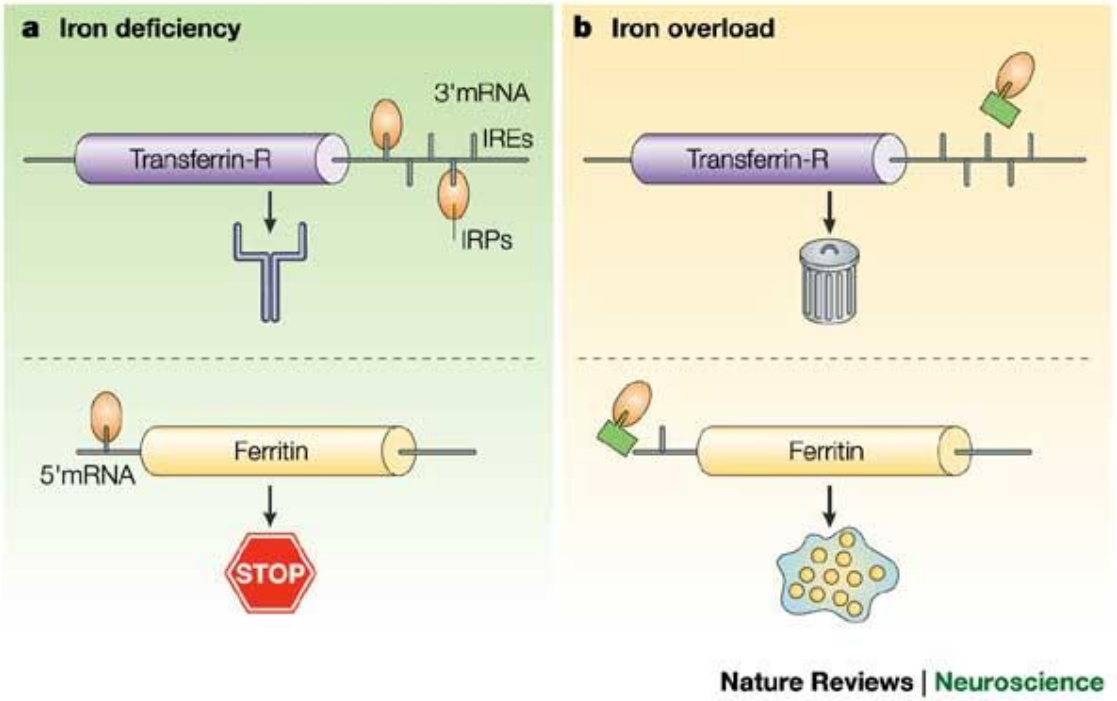
If iron is excessive IRE-BP will bind to it, and thus IRE-BP won't bind to the element on the mRNA of ferritin or on the mRNA of transferrin receptor.

As the element exist on different locations on each mRNA, (on the 5' of mRNA of ferritin & on the 3' on the mRNA of the transferrin receptor), the binding of the IRE-BP has different effect on both mRNAs.

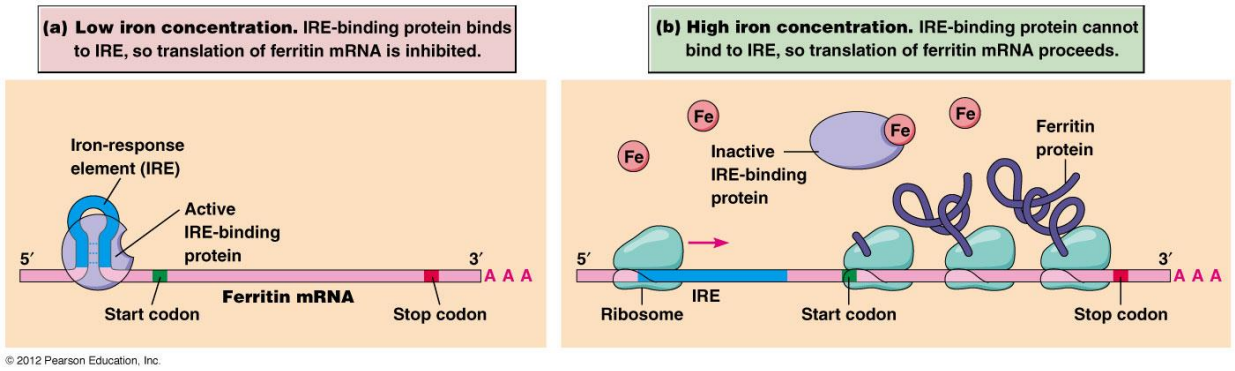
So when IRE-BP binds to iron, the mRNA of the transferrin receptor will be unstable and will undergo degardation. So the cocentration of the transferrin receptor protiens decreases.

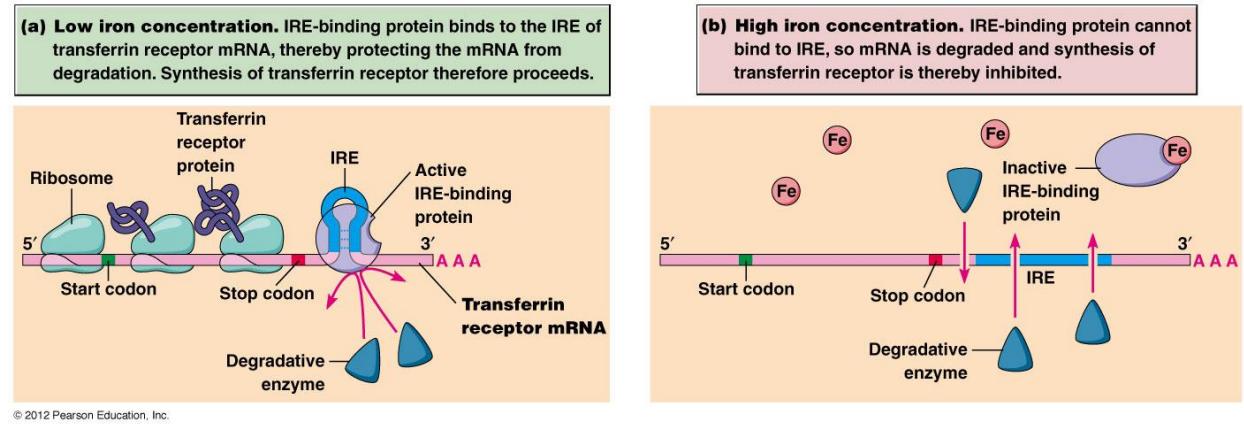
On the other hand, the IRE-BP unablity to bind to the 5' element of ferritin allows ribosomes to bind to the mRNA translating it and thus increasing the ferritin concentration. Therefore, the iron itself causes the cell to produce more iron storage molecules

If cells need iron=> the IRE-BP would be able to bind to the elements stablizing the mRNA of the transferrin receptor so the transferrin receptor will be produced. And preventing ribosomes from binding to the 5' end of the ferritin mRNA inhibiting it's translation and decreasing the ferritin concentration (opposite effect).



For better understanding: -





Remember ‘‘comfort is a beautiful zone, but nothing ever grows there”

Bhanu pratap singh.