Molecular Biology

Doctor 2019 | Medicine | JU

◉ Sheet ○ Slides

Done By

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Contributed in the Scientific Correction

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Contributed in the Grammatical Correction

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ملاحظه مهمه....

المحاضره هاي حكى الدكتور انها معلومات عامه للي بحب يستزيد

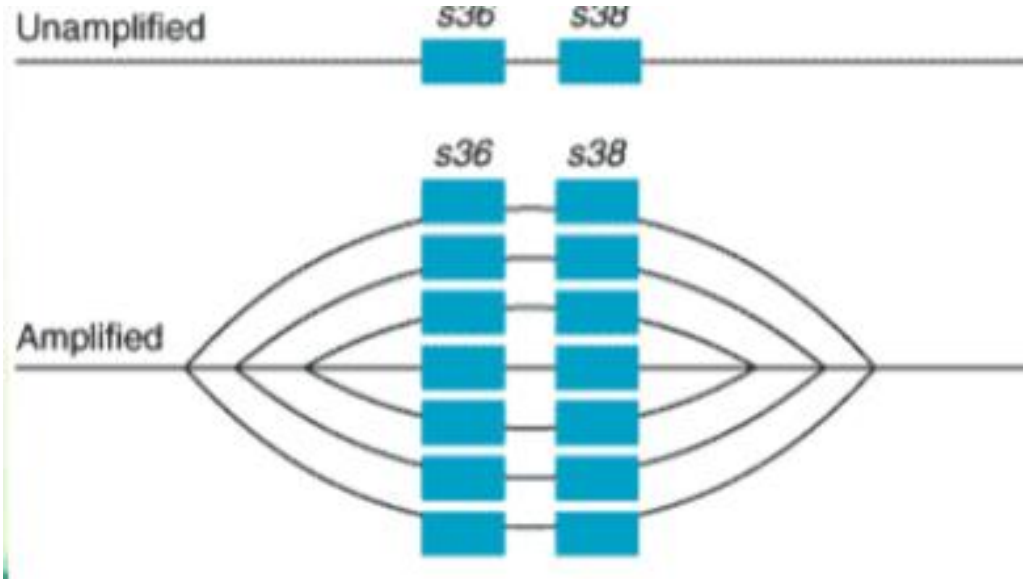
وحكى عل اغلب ما بيجي عليها سؤال ولو اجا رح يكون سؤال عام عن فهم

Short Introduction

There are some debates about some hypotheses in molecular biology
One of the hypotheses is RNA world hypothesis
Some scientists believe in it and some don't
It's not a fact
The central dogma of Molecular Biology is:
DNA -> mRNA -> RNA
mRNA is in the middle between RNA and DNA
RNA can be reverse transcribed into DNA
So some scientists believe RNA is the basis of life
One of the reasons it's that it can be used to make DNA, and it can fold which give it functional properties Like ribozymes (RNA molecules that fold and function as an enzyme)

Gene Amplification:

Gene amplification is an increase in copy number of a restricted region of a chromosome increasing the quantity of DNA in these regions without increasing the other gene
This happens as an error in DNA replication or DNA repair
In this chromosome puff we have a bigger number of genes than the rest of the chromosome (for a specific gene)



What is its importance?

First example

In some cancers we have overexpression of dihydrofolate reductase enzyme which is involved in cell growth and proliferation

Methotraxate works as an inhibitor to dihydrofolate reductase enzyme

dihydrofolate reductase is amplified when Methotraxate is used

Cancer cells use this to induce resistance against chemotherapy

Cancer is uncontrolled growth of cells

Cancer adapts signalling pathways to induce resistance and increase cancer cells

That's why more than one therapy is used for cancer

And cancer depends on (personalized medicine) in which genetic profiling is done for each patient and specific drugs are chosen that would work best for the patient

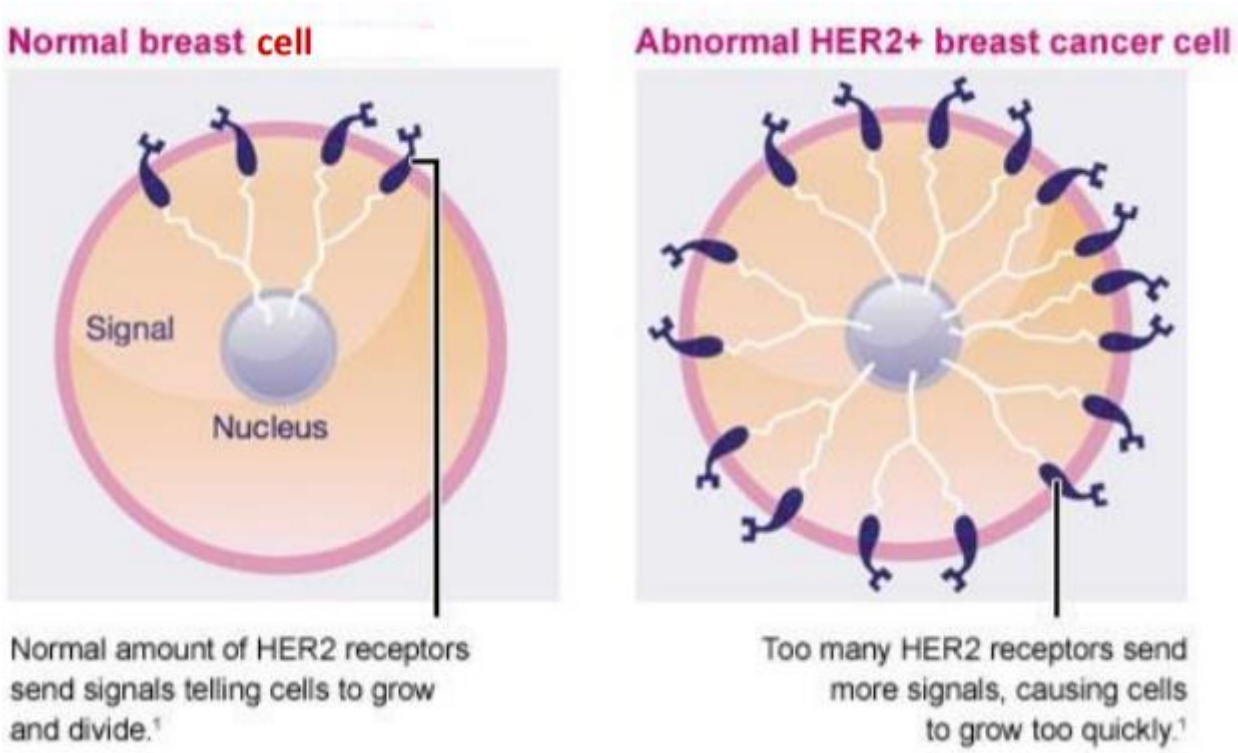
"The hallmarks of cancer" is a very informative paper that you can read if you want more information

Second example is human epidermal growth factor receptor 2 (HER2)

(tyrosine kinase receptor) , this receptor is overexpressed in about 30% of breast cancer cases

And overexpression is mediated by gene amplification

In this image we have normal cells and breast cancer cells (look at the difference in the number of receptors)



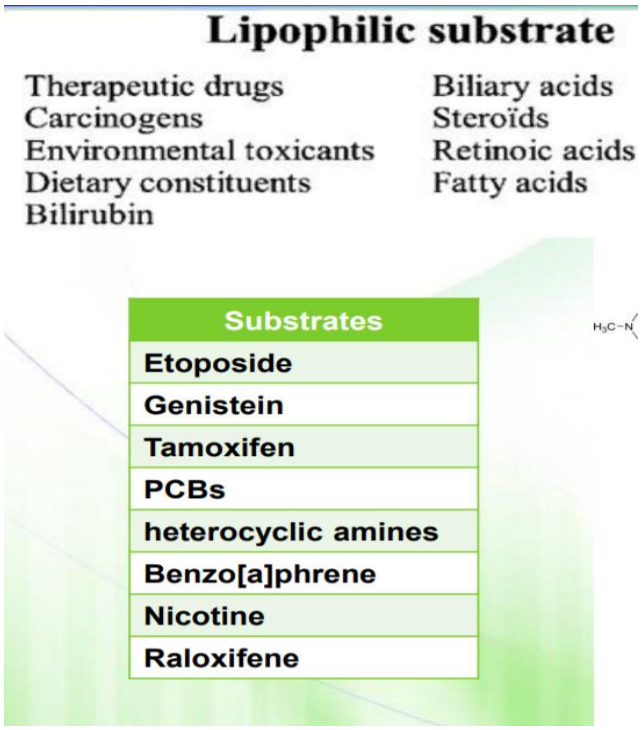
But we have drugs (herceptin) (antibody) which can inhibit the pathway of HER2

Overexpression can be associated with other mechanisms like the mutations that activate transcription factors that can cause in the amplification of a given gene

Now let's talk about another phenomena which regulates gene expression in eukaryotic cells which is the use of multiple promoters or alternative promoters.
We talked alternative splicing which is the splicing of pre-mRNA to produce different combinations of exons which produce different mRNA molecules which produce different proteins which are called protein isoforms

Another mechanism which can produce spliced variants is the use of multiple promoters or alternative promoter
You might think that 1 promoter regulates pre mRNA which is spliced to make different proteins
This mechanism makes spliced variants
One example is an enzyme that's called UDP-glucuronosyltransferase (UGT)
The uridine diphosphate glucuronosyltransferase (UGT) enzymes transfer of glucuronic acid into xenobiotics and lipophilic (hydrophilic) and other endogenous compounds making them water soluble and allowing for their biliary or renal elimination.

UGT is a family of enzymes that are responsible for glucuronidation of hundreds of compounds.
Examples of lipophilic molecules(مش للحفظ)



UGT is responsible for the conversion of these molecules into water soluble molecules to allow the secretion of these molecules from our body
The glucouridination can occur in different tissues
Glucuronidation of tamoxifen can occur in the colon , intestines .
But nicotine happens in the breast, colon, esophagus, ovary, prostates, and testes.

Substrates	Place of reactio
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

What causes the difference in tissue place?
The UGT contains fixed catalytic subunits that do glucuronidation reaction and at the same time has different substrate binding sites that bind different molecules.
For example drill has one function but you can add different pieces
And one head can take different hats
So different substrates->same function
Different substrate binding sites come from exons that provide diversity
But the catalytic subunit is fixed.

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 and **each with its own promoter**.
The 9 exons determine substrate specificity and one of them is spliced to an exon generating 9 possible UGT1A transcripts.
Basically exon 1 encode the different substrate sites

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

Last thing is

Regulation of mRNA stability

You remember that mRNA is degraded and in prokaryotes mRNA is less stable

So how does mRNA stability control the expression of proteins?

An example is iron responsive elements (IRE)

(الشرح الي تحت من شيت 018 ... صدقا شفته افضل واحسن)

*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.

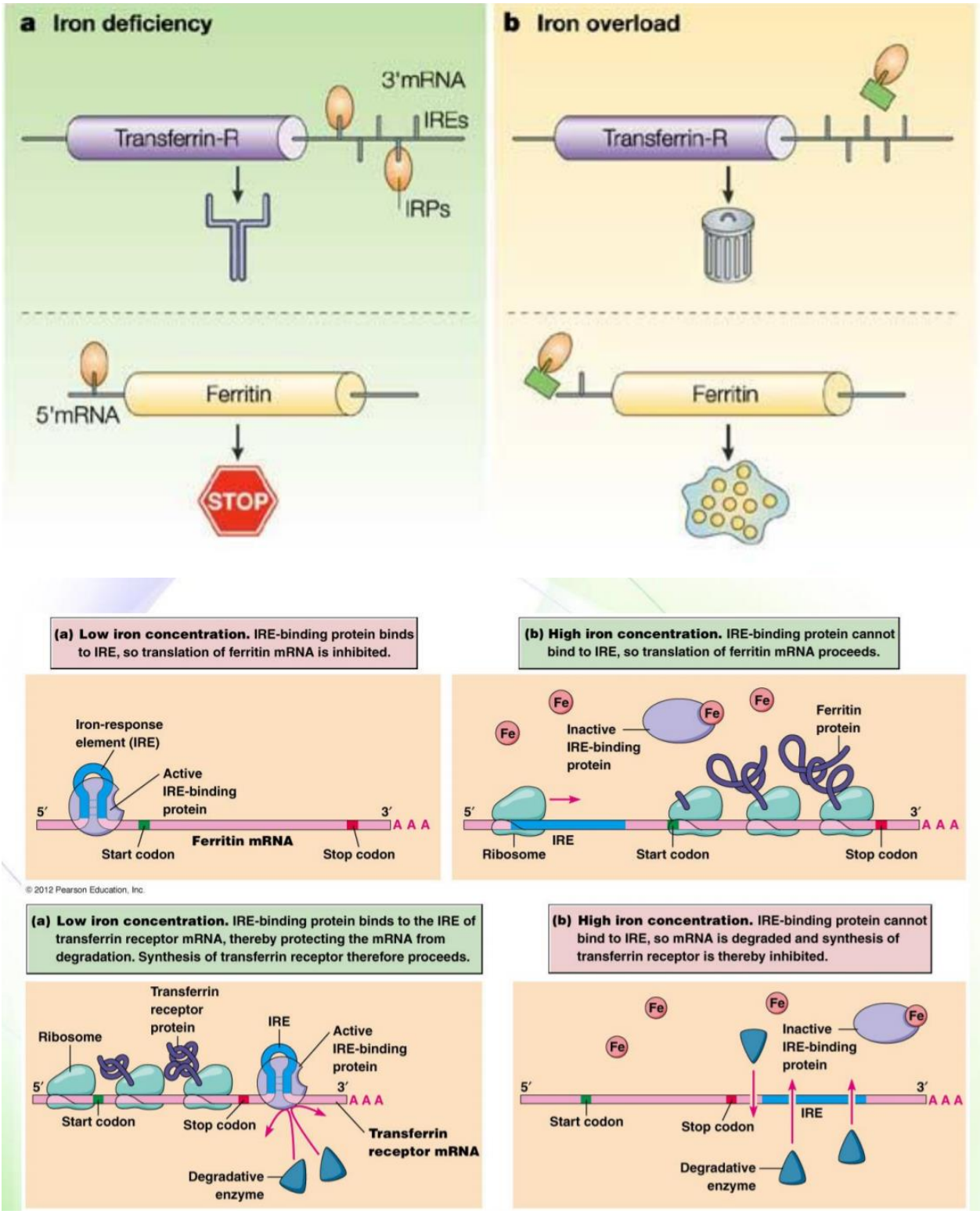
A single Ferritin has the ability to bind to 4000 iron atoms storing them.

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 increase
If cells don't need iron (there's an excessive amount of it)→ ferritin is made
.

So, in the body the enzymes that have opposite functions are often regulated by the same mechanism that has a 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).


a Iron deficiency
3'mRNA
IREs
Transferrin-R
IRPs
5'mRNA
Ferritin
STOP
b Iron overload
Transferrin-R
Ferritin
(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.
Iron-response element (IRE)
Active IRE-binding protein
5′
3′
A A A
Ferritin mRNA
Start codon
Stop codon
Fe
Inactive IRE-binding protein
Ferritin protein
Ribosome
IRE
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(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.
Transferrin receptor protein
Ribosome
IRE
Active IRE-binding protein
Transferrin receptor mRNA
Degradative enzyme