

Today our lecture is about regulation of gene expression at the translational level and post translational level.

- **Regulation at the translational level** : means regulation at the protein synthesis level

There are some examples mentioned regarding this level:

1) Control of initiation factors (IF):

As in maturing RBCs, why aren't we talking about mature RBCs? Because the mature ones don't have DNA & thus no transcription occurs there.

At the final stages of their maturation, RBCs get rid of most of their genes & only keep one gene coding for one protein which is the globin, for the sake of making hemoglobin... but still, the RBC is economic in this process; i.e. the RBC doesn't make too much globin if it doesn't have enough heme for it... it only makes globin in amounts equivalent to the heme amounts the RBC has.

⇒ No heme no globin protein synthesis.

Now, how does the RBC know the amount of heme present inside it?

Let's go back to the initiation stage of translation, where there was assembly of the translation complex on the mRNA (large & small subunits, mRNA & the first tRNA) by initiation factors (*factors that are responsible for the formation of the translation complex in the translational level so if these factors stop functioning, the whole translation process won't occur*)... most importantly... eIF2 (eukaryotic Initiation Factor 2).

Now, if we control eIF2, we'll control the translation of the globin gene, how does this happen?

eIF2 is a phosphoprotein; which means that once it's phosphorylated, it becomes inactive & vice versa. An Enzyme that is responsible for the phosphorylation of eIF2 is heme kinase, which is regulated by heme, i.e. when there is enough or even high heme conc., this will inhibit heme kinase so it is unable to phosphorylate eIF2 so it will be active (in the dephosphorylated form) and then, eIF2 is able to assemble ribosomes on mRNA and the formation of globin occurs and vice versa.

Remember: the inactive form of eIF2 is the phosphorylated form (eIF2_P)... whereas the active form of eIF2 is the dephosphorylated form.

Conclusion:

High heme conc. will increase globin protein synthesis.

Low heme conc. will decrease globin protein synthesis.

2) Ferritin synthesis :

Function of ferritin is iron storage in cell.

Also here, the cell is economic; if it doesn't have high amounts of iron, then why to synthesize more ferritin than needed? ... More iron, more ferritin... less iron, less ferritin.

In human cells, there is a loop – hair pin loop - near the 5' end of the ferritin mRNA called iron responsive elements (IREs).

Why do these loops happen? Due to the presence of complementarity between 2 regions of the mRNA –the tips of the loop-.

Some of the loops on mRNA are sites for binding of specific proteins, this loop – IRE – has a specific protein that binds to it which is - IRE binding Protein -.

In the slide 217:

The picture shows mRNA for ferritin on the 5' end of it there is a loop called Iron response element (IRE).

_AT low iron levels \longrightarrow Iron response element binding protein (IREBP) binds to IRE on the 5' end of ferritin mRNA, this binding will stop the ribosomes from translating the mRNA & thus blocks translation process so no ferritin synthesis occurs.

_At high iron levels \longrightarrow Iron binds to IREBP since it's the NATURAL ligand for IREBP & it has a higher affinity to it than IRE & then iron removes the IREBP from IRE on the ferritin mRNA, so by this, ribosomes are now capable of translation and ferritin synthesis; since the loop – IRE – itself can't stop the ribosomes from functioning on the mRNA...

3) Stability of mRNA

NOTE: Ferritin (a protein that stores iron), Transferrin (a protein that transports iron in blood and binds to the transferrin receptor on cell surfaces), transferrin receptor (present on cell surfaces & in case the cell needs iron, it'll bind to transferrin to take iron from it to the inside of the cell).

So when the cell needs iron, it'll synthesize transferrin receptor & expresses it on the cell surface to bind to transferrin & brings in iron.

Again this process is controlled by iron levels & this is how it happens:

IRE loop is in this case present on the 3' end of transferrin receptor mRNA (not on the 5' end)

_ if there is low conc. of iron:

IREBP binds to IRE on the 3' end of the transferrin receptor mRNA and this will prevent degradation of mRNA by RNAases and this binding results in increased mRNA half life & so increased protein synthesis.

NOTE:

Binding of IREBP on IRE at the 3' end won't affect the translation process or ribosomal function (translation occurs from 5' to 3').

_ if there is high conc. of iron:

In this case, iron binds to IREBP preventing it from binding to the IRE on the 3' end on mRNA of transferrin receptor, resulting in exposed 3' end region in front of the RNAases which leads to decreased half life and degradation of the mRNA so no transferrin receptor synthesis & no uptake of iron occurs.

4) MicroRNA (miRNA): "RNA interference"

It was first discovered in nematodes, then was found in plants, animals & finally in humans, in which the cells regulate gene expression at the post-transcriptional & translational levels through it.

If it leads to mRNA degradation, then it's regulating at the post transcriptional level BUT if it prevents translation, then it's regulating at the translational level.

MiRNA is processed in the cytoplasm through binding to 2 complexes, the first is DICER, which processes the miRNA & forms a complex out of it, then, this complex binds to the mRNA, after that, the second complex – RISC – is activated & it'll break the mRNA very fast.

What does the miRNA do?

If the cell has too much of a certain protein, then the mRNA copies of this protein are no longer needed in the cell, so there is a need to break down (degrade) those mRNAs immediately without waiting for the action of fast RNAases, how is that? Each unwanted mRNA will be labeled with miRNA that is complementary to a specific region on the mRNA; this miRNA strand will act as a marker for accelerating destruction, which leads to the activation of a complex called RISC which degrades mRNA very fast.

NOTE: miRNA is very short, having (20_22) nucleotides which are complementary to a specific region on the mRNA needed to be degraded ...

If the miRNA that binds to mRNA is 100% complementary (high homology), this will lead to degradation of the mRNA, whereas if there is poor homology between the two (not 100% complementary) this will lead to inhibition of translation (i.e. mRNA isn't degraded). Eventually in both cases the mRNA isn't translated.

Until now they discovered 1000 miRNA controlling about 1000 gene & some of these miRNAs are found in the non-coding region.

Genetic Engineering Application on miRNA:

The usage of miRNA in cells is a natural mechanism to down-regulate certain genes... & this was the principle used in the following application:

In the past, if scientists wanted to know the effect/ function of a specific gene, they used to remove it from the embryonic mouse -so that when it grows, it won't have that gene at all- & see what happens to that mouse later in life & this is called **gene knockout**; for example, if the mouse turned up to be blind, then the gene isolated is responsible for vision & so on... but the gene knocking-out process is so difficult; since you are removing a gene which isn't easy...

Now, there is what is called **gene knocking-down**; where the scientists introduce miRNA - siRNA (small interfering RNA) - that breaks down the mRNA of the gene - they are studying its effect – all the time, no matter how much the cell expresses that gene, all the mRNA copies will be degraded & through this while of miRNA action, scientists can actually examine the mouse to see what happened upon knocking down that gene... the special thing about this procedure is that once scientists are done studying that gene & know its function, they can stop the miRNA & the gene will be expressed normally... As if when the gene functions back, you'll be a 100% sure that your studies were true, because you can

see the effect when the gene is on & compare it with the effect you concluded of the same gene while knocking it down.

Also, miRNA is now used in the treatment of cancer, some cancers result from oncogene over-expression, so, miRNAs are now designed to bind to mRNAs of these oncogenes... in this case, when you design a miRNA that isn't present inside the cell, it is called siRNA (small interfering RNA).

*Remember, more than one miRNA might bind to one mRNA -each time a different miRNA-, and one miRNA might bind to 2 or 3 mRNAs.

Regulation at the post-translational level: i.e. the protein is already synthesized, but here we are controlling essential protein modifications which will control its activity, such as:

- 1- **Polypeptide processing** (e.g. folding, cleavage like in insulin, pepsinogen...)
- 2- **Conjugation** : e.g.

-Glycosylation

-Phosphorylation for quick regulation, i.e. add a -P-group for activation, remove the -P- for deactivation and in some cases vice versa.

** (most of the transcription factors are phosphoproteins)

-Methylation

- 3- **Degradation:**

- E.g. ubiquitination which is adding ubiquitin ligand to the protein that is meant to be degraded, which is considered as a marker for proteases for degradation.

- 3- **Localization:**

Simply, if there is a nuclear protein present in the cytoplasm and we want it to function, it will be internalized to the nucleus, if we no longer need it, it will be transported to the cytoplasm... as in glucocorticoid receptor.

CHAPTER 18: CANCER MOLECULAR GENE

Cancer: uncontrolled cell proliferation

Characteristics of cancer cells in vitro (in tissue culture dish):

-No contact inhibition; in normal cell culture, the cells grow & proliferate until they form a complete monolayer & upon adhesion/ contact between cells, there will be a sort of messages between the cells to stop proliferation ending up in monolayer formation, in cancer cell culture, the cells grow & proliferate & there will be no messages to stop the growth upon contact between the cells, ending up in multilayer formation.

-No need for stimulation by growth signals.

-Resistant to inhibitory growth signals.

-Resistant to apoptosis, i.e. they are immortal cells that don't even become senescent.

-Loss of anchorage dependence. اذا جيت خلايا طبيعية و اعطيتها هيكل العضو، رح تنمو عليه وبتاخذ شكل العضو (عندها ذاكرة)، اما الخلايا السرطانية اذا اعطيتها هيكل العضو اللي جاي منه من الجسم، رح تنقسم بشكل عشوائي، ما عندها ذاكرة.

- Form tumors in nude mice, nude mice are mice without immunity, so they won't reject a human cell & if are injected with a normal human cell, it'll eventually die, whereas, if they were injected with a human cancerous cell, it will form a human cancerous tumor inside these mice... why do scientists do all that? To test the malignancy of cells by putting them in these nude mice.

-Benign VS malignant tumor (invasive vs non invasive)

- Invasion: penetrance through the boundaries of the tissue the tumor cells are residing in.

- If the cell doesn't invade the adjacent tissue it's considered as benign tumor if it invaded the adjacent tissue it's considered as malignant tumor.

The nevi is a tumor, its cells divided and stayed at the same site (did not invade the epidermis and the dermas and didn't reach the blood or the bone)... so here it's a benign tumor.

Now, if this nevi was the beginning of melanoma (skin cancer), after few months, it will invade the epidermis and the dermis and reach the blood and the bone & here it's malignant.

-The malignancy can be metastatic or not.

The metastasis means the movement/ migration of the cancer cells through the blood or the lymphatics to other places and this migration results in the Formation of a secondary tumor.

-People thought that metastasis happens randomly but in real it's a very well orchestrated process, for example the cancer cells which are found in the breast send Scouts (خلايا كشافة) those scouts will circulate in the blood and they will finally choose one organ with a good environment, for example they will choose the lungs in case of breast cancer. .

Then, they will start forming a nest within the lung (nesting), then they will send signals to the

cancer stem cells (the most malignant part of the tumor) to come to the lung, those stem cells will go to the lung and implant themselves there forming the secondary tumor.

So the metastasis is no longer thought as a passive random process.

Carcinogens: anything that produces a cancer (chemical, physical, viral)

Chemical: any substance that interferes and reacts with the DNA and produces mutations.

Radiation: is not a reaction, it's an energy that penetrates our body and our cells & nuclei in them, resulting in destruction/ breakage in our DNA.

Tumor viruses (retroviruses): produce cancer if they interfere with a gene that is associated with cancer, resulting in mutagenesis & carcinogenesis.

**All of our oncogenes are found in viruses

Genetic predisposition:

-cancer is a genetic disorder produced by a mutation on the level of the gene but its not a hereditary disorder, but sometimes, we may inherit the Susceptibility to develop a certain cancer, which means in this case that we are highly predisposed to that cancer (breast and colon cancer).

-Cancer is not produced from one mutation in one oncogene, cancer will be produced by the accumulation of mutations (4-7) some books say (5-10), those mutations are necessary for full transformation.

Oncogenes and Tumor suppressors (the most important part) :

Oncogenes :

The oncogenes in their normal situations are called proto –oncogenes, and they are responsible for normal and limited cell division (regulated) , if the activity of those genes increase (leading to abnormal and unlimited cell division) , then those proto–oncogenes become oncogenes.

Tumor suppressers:

genes that prevent cells from division ضوابط الانقسام

If we have a car we will consider the speeding pedal as the proto-oncogene so this proto-oncogene will allow the cell to divide once it's needed the same as the speeding pedal.

If you press the pedal without a control you will end up with an accident same as the proto-oncogene the over activity of the proto -oncogene results in more divisions, more mutations and finally the development of cancer.

The tumor suppressor genes represent the breaks of the car if the cell starts to divide abnormally those suppressors will prevent the cell division and also the car breaks, they prevent the car accident.

So the cancer contains 5-10 mutations with up regulation of the oncogenes and down regulation of the tumor suppressors.

The up regulation of the oncogene needs only activation of one copy of the oncogene, whereas the down regulation of the tumor suppressors needs inactivation of both copies of the tumor suppresser gene مثل سيارة التدريب

This information is very important for the understanding of the familial cancers and the predisposition to them.

Faith is the first factor in the life devoted to successwithout it nothing is possible with it nothing is impossible.

..... just smile

forgive me for any mistake

GOOD LUCL =D