17

Molecular Biology

Doctor 2019 | Medicine | JU

Sheet Slides

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ملاحظة صغيرة: كل الي مكتوب بالأسود هو الكلام الي موجود بالسلايدات أما الملون (الزهر الفاتح والخمري) هو كلام الدكتور, موفقين جميعا

Translation Part 2:

-Topics of this lecture: in this lecture we'll talk about: **stages of translation**, **protein factors that are involved in the translation process**, **regulation of translation** and **some models that regulate synthesis of proteins**.

Now let's start our lecture

Building a Polypeptide:

-Translation contains **three stages**:

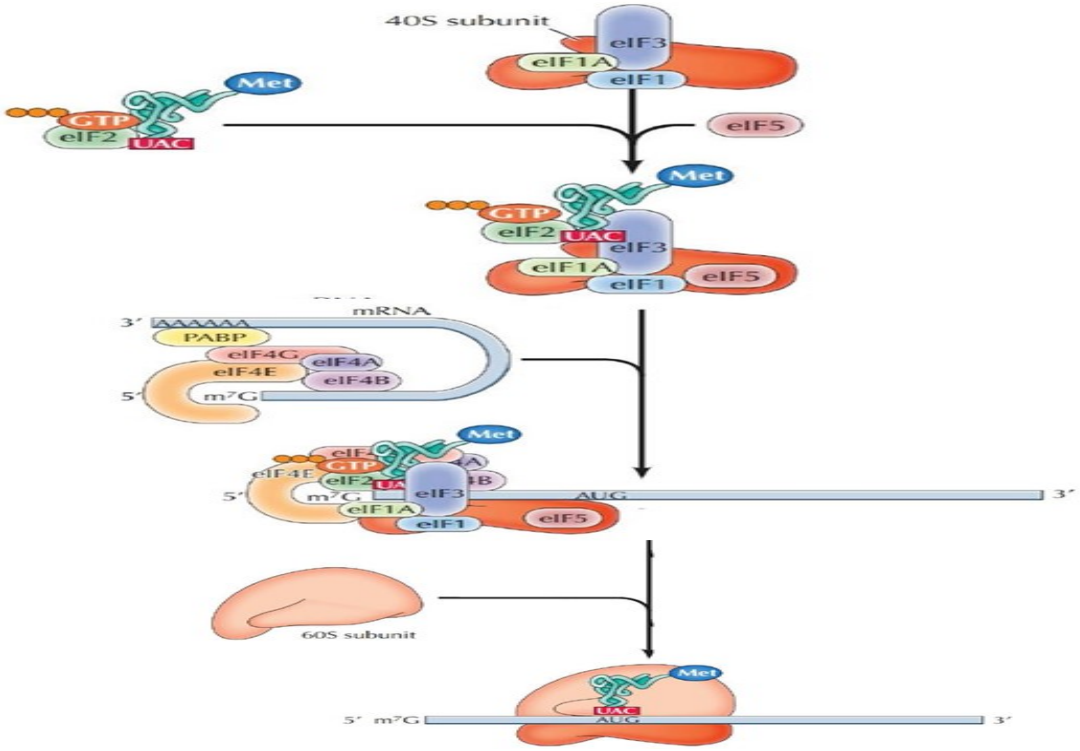
1- Initiation **2-** Elongation **3-** Termination

All three stages require protein "factors" that aid in the translation process (these protein factors help in regulating this process)

Translation initiation:

-Remember that we have a start site in the mRNA which is **AUG** codon, a codon for **methionine** amino acid.

-About this figure:



1-tRNA forms a complex with 40S ribosomal subunit: at the beginning the tRNA that carries the methionine amino acid should assemble to the 40S ribosomal subunit (the small ribosomal subunit)

2- mRNA joins the complex (tRNA complex)

3- After that, the 40S ribosomal subunit scans for the first AUG.

4- The large ribosomal subunit joins them all (the small subunit and mRNA) to start the translation

#During all these steps we have protein factors that aid and help to regulate translation initiation (A large group of initiation factors facilitate every step), in this example eIF2 (eukaryotic initiation factor 2) which is very important in bringing the tRNA with the methionine amino acid with the small ribosomal subunit (eIF2 brings tRNA to small ribosomal subunit)

-Also, we have **eIF2** and **eI4G** which play very important rule in bringing mRNA to tRNA/40S ribosomal subunit. **(NOTE: you just need to know the general factors involved 'those mentioned in the sheet' because it's very complicated)**

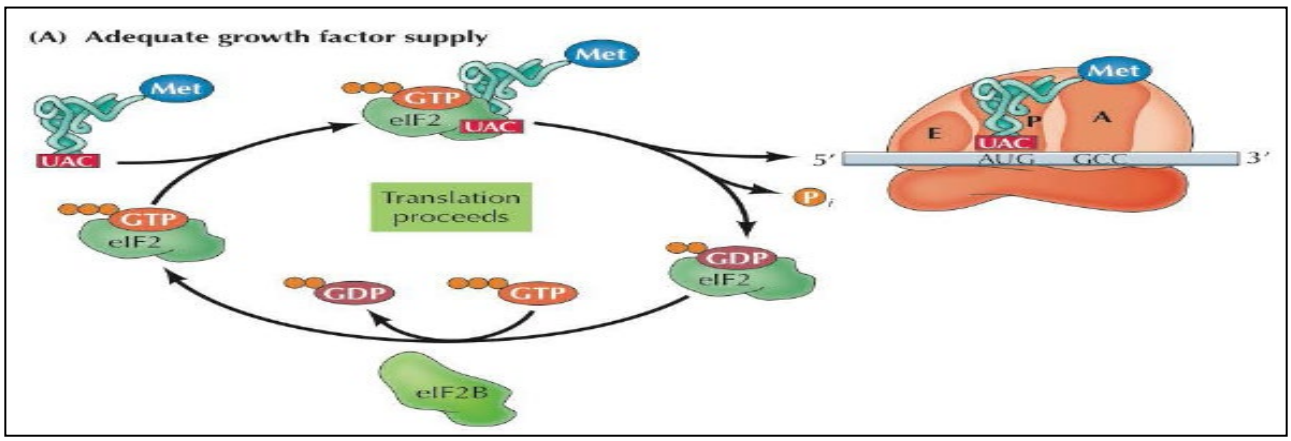
Regeneration of eIF2:

ملاحظة:في خطأ طباعة بالسلايدات معدل بالفيديو لهيك حيكون مزبط بالشيت متل ما هو بالفيديو

-Important information: eIF2 can be regenerated and recycled so it can be used several times in translation initiation.

At the beginning eIF2 should be complexed to GTP to be active, and then when the correct tRNA (with methionine) is inserted, GTP is hydrolyzed to GDP. At this moment the GDP will be dissociated from the eIF2and the **eIF2 will be inactive in this stage.**

After that the eIF2 will bind again to new GTP and becomes active, the active eEF2/GTP complex must be regenerated by exchanging of the bound GDP for GTP, and this cycle can be repeated several times.



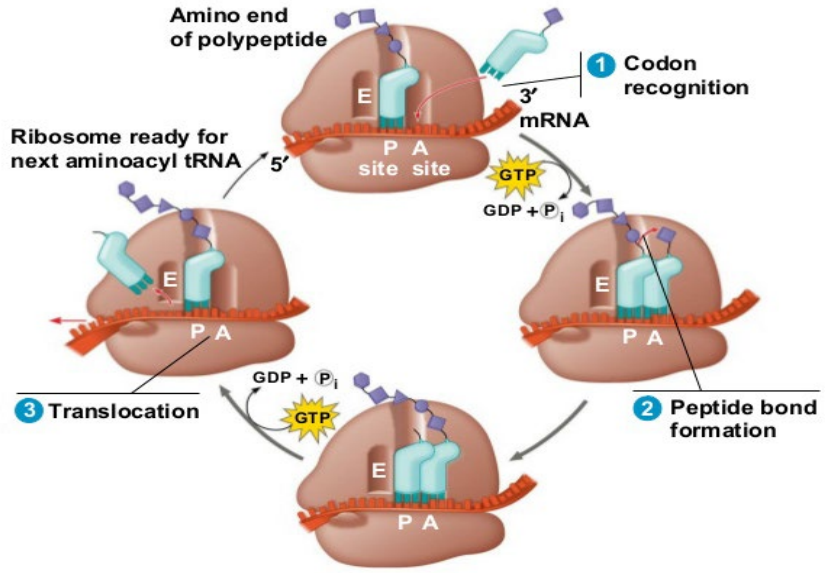
Translation elongation I:

It contains three steps:

1-Aminoacyl-tRNA binding: aminoacyl-tRNA bind to the A site.

2- Peptide bond formation: between the growing polypeptide (the carboxy terminus) and the coming amino acid (N- terminus)

3- translocation with the help of elongation factors (eEF): the (P) and the (A) sites are translocated, and uncharged tRNA will exit the ribosomes so it can be used for another amino acid and the growing polypeptide linked with tRNA stays in the (P) site.



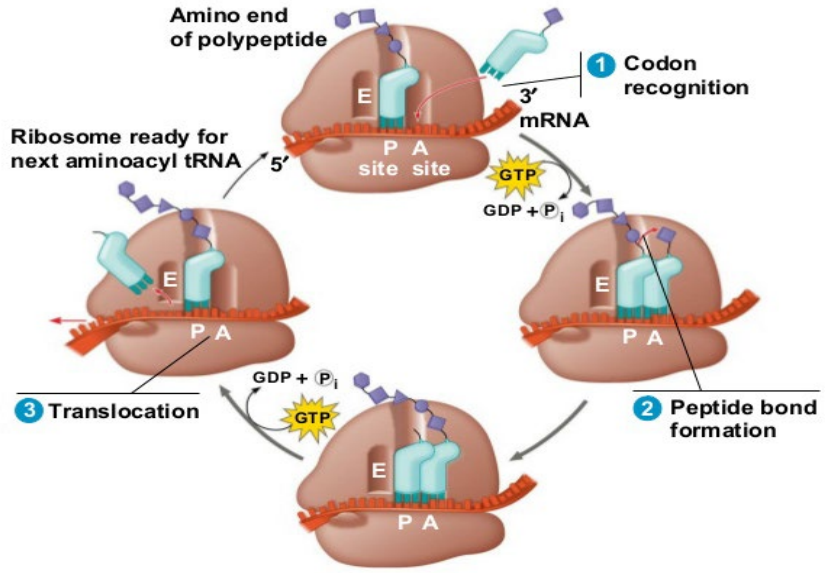
-So as the translation occurs, the polypeptide chain draws again and again.

We have different eukaryotic elongation factors so you need to distinguish between initiation and elongation factors, so for now we have:
1- eEF1α brings next aminoacyl-tRNA to the A chamber in the ribosome
2-eEF2 that is critical in ribosomal translocation (tRNA translocation and the exit of uncharged tRNA from the ribosomes)

Steps of elongation in more details:

1- First the new amino acyl tRNA will come and bind to the (A) site and in the A site we have the codon that is complementary to the anticodon

2- After binding, a peptide bond will start forming between the growing polypeptide (carboxy



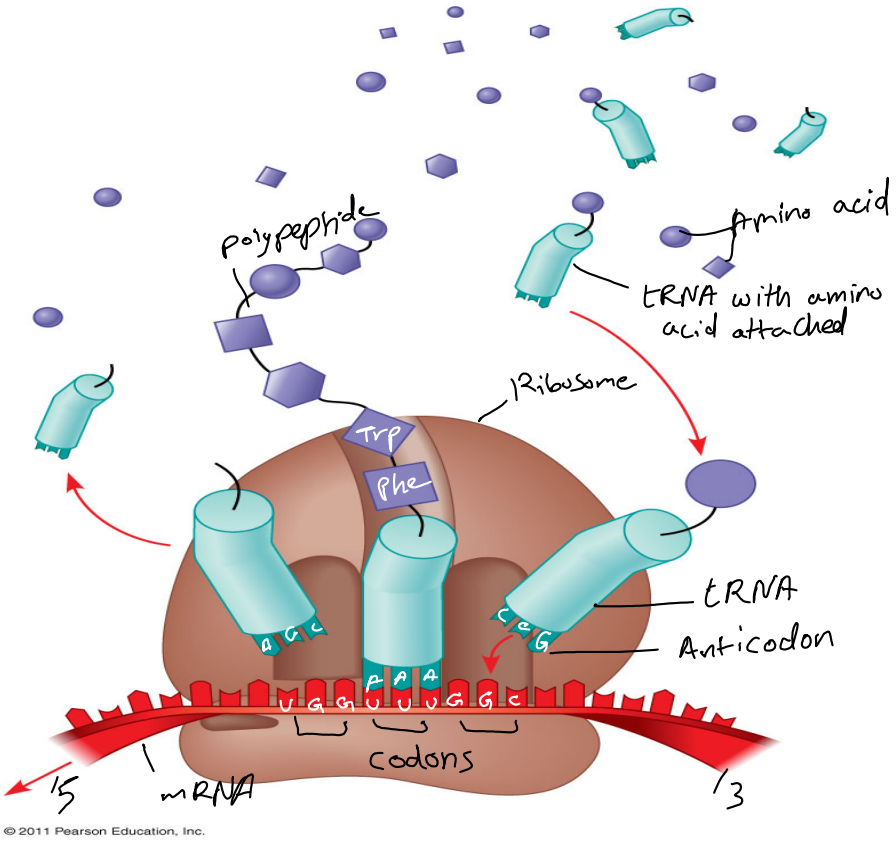
terminus) and the coming amino acid (N- terminus).

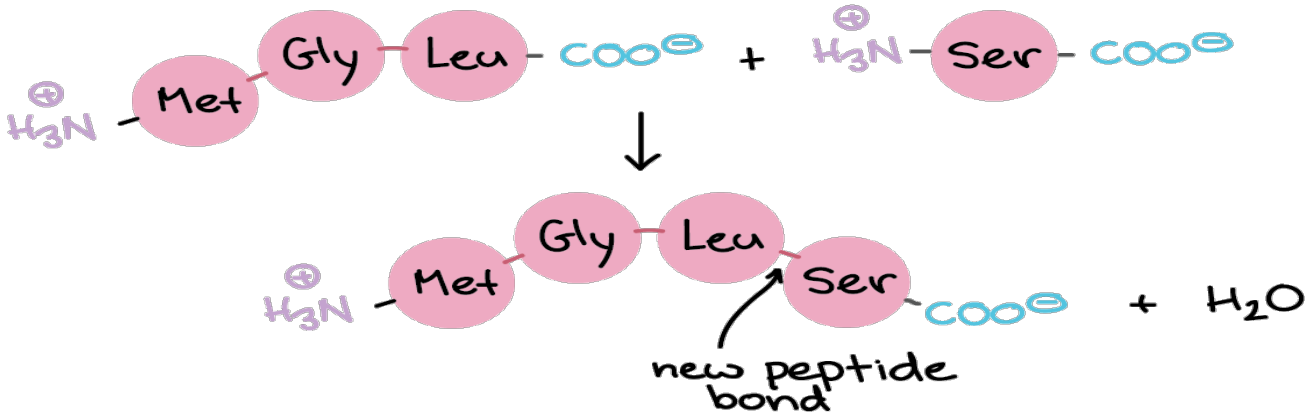
3- After that, the tRNA in the (A) site will catch and carry the polypeptide and the tRNA in the (P) and (A) sites will be translocated. **So, the uncharged tRNA will be removed from the ribosome through the exit site and the tRNA in the (A) site will be translocated to the (P) site, *and now the ribosome is ready for the next amino acyl tRNA* to come and so on.**

-The next figure summarizes the elongation process

Remember that reading of mRNA is from **5'** to **3'**

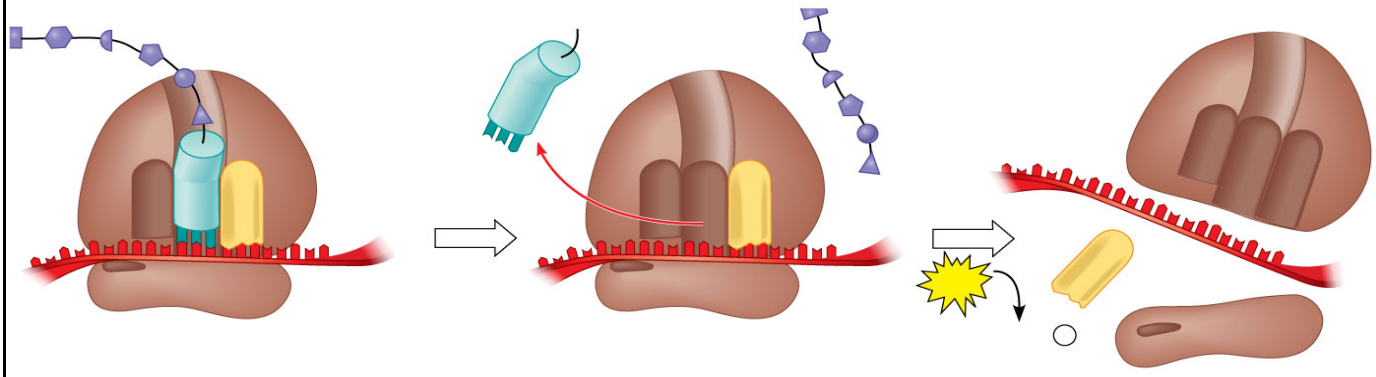
-you can see the entrance of the aminoacyl tRNA, binding to its codon in the (A) site, and then the formation of a peptide bond between the growing polypeptide and the new amino acid, then the translocation of tRNA in the (A) and (P) sites and the exit of the uncharged amino acid (actually the uncharged amino acid will be again charged and used in the next protein synthesis)





During the elongation stage, amino acids are added one by one to the preceding amino (N)-terminus (of the coming tRNA) to the carboxy (C)-terminus of the growing chain.

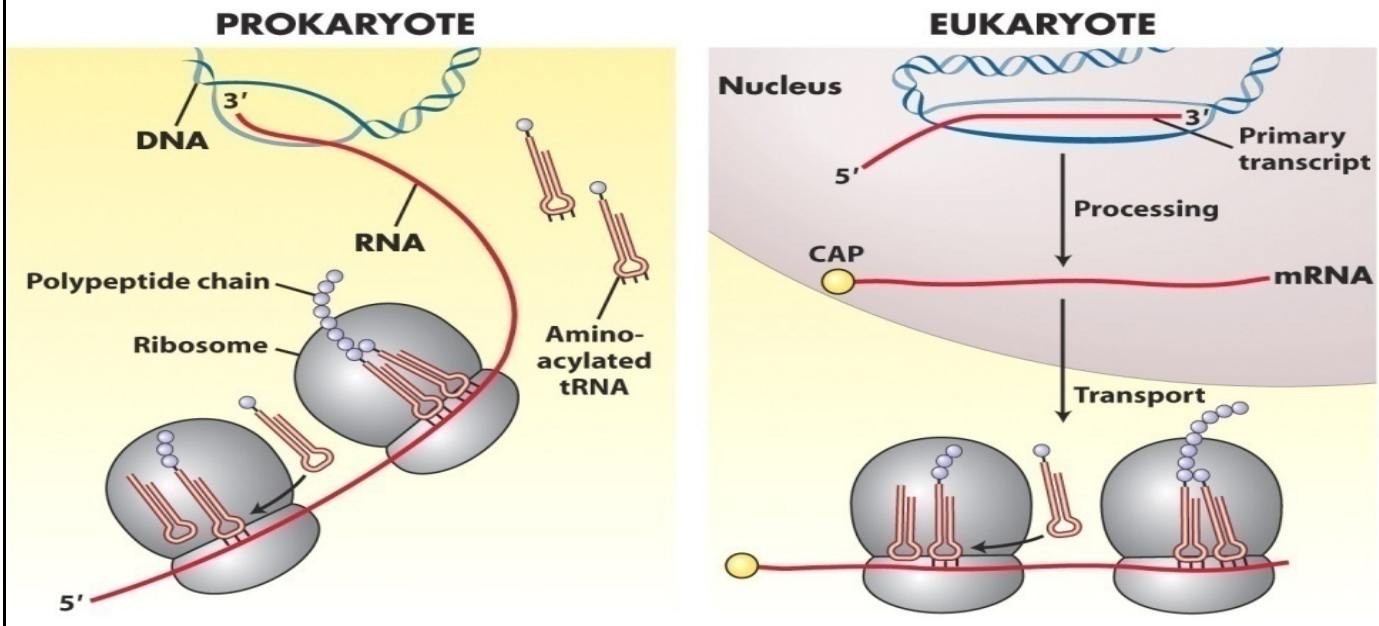
Termination of Translation:



-It is the last stage of the translation process, so we need a signal that will tell the machine to stop and that there's no more synthesis of proteins. The codons UAA, UAG, and UGA are the stop signals (when the ribosome reaches one of these codons, that's a signal for termination). They are not recognized by any tRNAs (because they are stop codons and not codons for any amino acid), but a release factor protein.

The A site accepts **the release factor,** which causes the release of the polypeptide, and the translation assembly then comes apart (the release of the ribosomal units from the mRNA).

Transcription/translation Coupling:



-Remember that in prokaryotes there's no nucleus but it is present in the eukaryotic cells, so that translation and transcription are coupled in space and time in prokaryotes (occur at the same time and place), **BUT** it's not the same story for eukaryotic cells

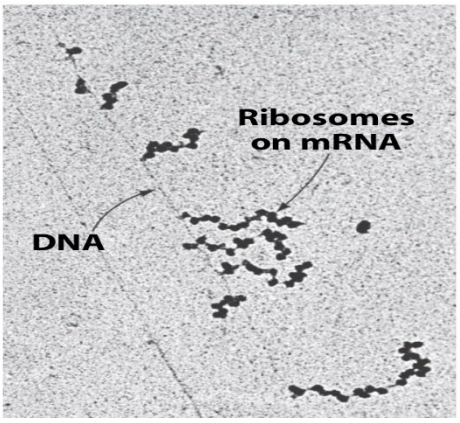
because the transcription occurs in the nucleus then the primary transcription undergoes processing to produce mRNA and this mRNA should be transported to the cytoplasm where the translation occurs.

-So, the transcription and translation are directly coupled in prokaryotes while they're indirectly coupled in eukaryotes.

Polyribosomes (polysomes):

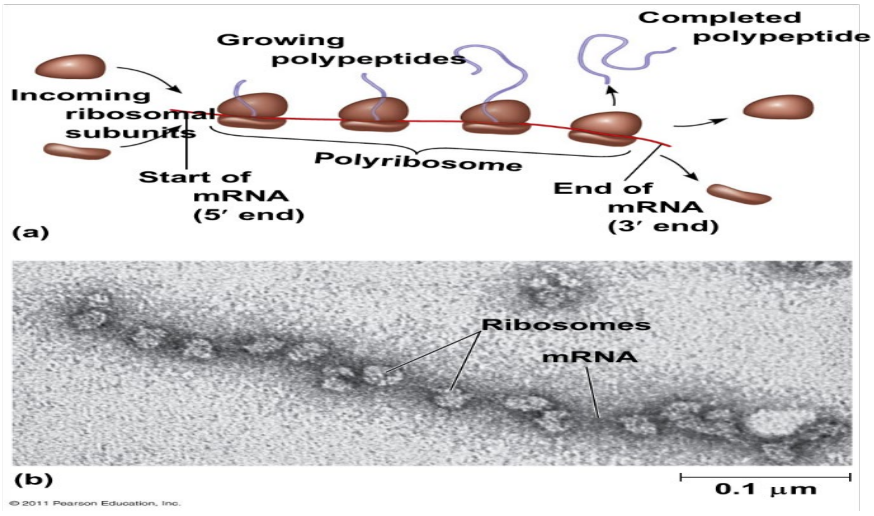
A single mRNA molecule is translated by several ribosomes simultaneously (simply polyribosomes used to describe the translation of a single mRNA molecule by several ribosomes simultaneously to produce several copies of the polypeptide chain), and these polyribosomes exist in both prokaryotes and eukaryotes. Each ribosome produces one copy of the polypeptide chain specified by the mRNA. When the protein has been completed, the ribosome dissociates into subunits that are used in further rounds of protein synthesis.

-Here for example in this figure, which is for a **prokaryotic** cell, you see electron microscopy for the DNA sequence and we have several mRNA copies and we have several ribosomes bond to single mRNA molecule.



Polysomes (in eukaryotes):

We have a little difference between prokaryotes and eukaryotes cells by means of translation and transcription coupling, and in eukaryotic cells mRNA is located in the cytoplasm where the translation takes place. A number of ribosomes can translate a single mRNA simultaneously, forming a **polyribosome** (or **polysome**). Polyribosomes enable a cell to make many copies of a polypeptide very quickly.



A benefit of restriction endonucleases (cloning to produce of hormones):

We can use the cloning technique to produce recombinant proteins.

-We have many recombinant or therapeutic proteins in the clinic for therapy just like insulin for the treatment of diabetes, so we need synthesis of large quantities of these hormones and techniques out of our bodies for that. One of these methods is cloning of a specific gene into a plasmid (which is a circular DNA that is found in bacteria and it's responsible for the expression for several genes involved in the bacterial growth).

-Cloning is still common and used to produce proteins, **but the question now how can we use cloning to target a gene or specific DNA sequence into the plasmid?**

1- First we have a plasmid with restriction sites (in which restriction endonucleases enzymes bind and cut at these sites)

2-After cutting the plasmid we bring the DNA sequence of a target gene like insulin gene and we do ligation between the DNA segment of the gene and the plasmid, the target gene will be part of the plasmid

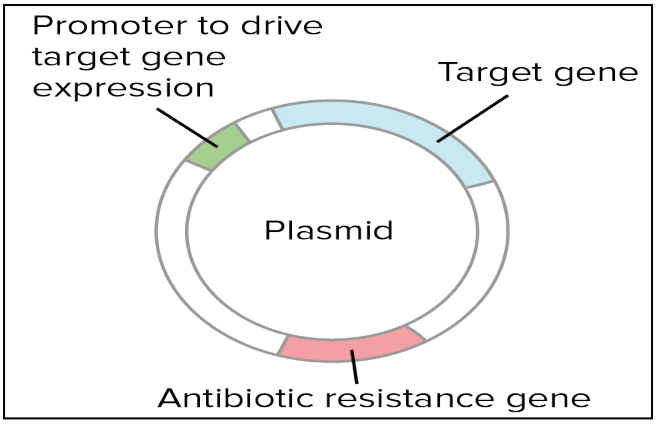
3-the expression of this gene will be regulated by a promoter that exist in the plasmid sequence

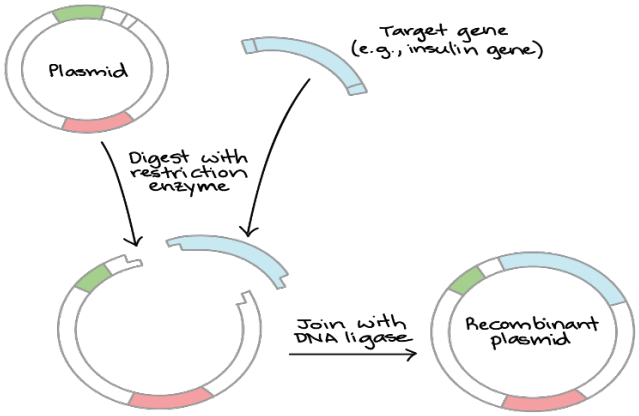
**the expression of genes in prokaryotes is very high and rapid producing many proteins and of course we have some methods for that in eukaryotes.

-The resulting cloned plasmid will transform into the bacteria so it would be delivered into the cytoplasm of the bacterial cells where the transcription and translation of the that cloned gene occur to produce enough amount of recombinant protein (produced protein).

-**VERY IMPORTANT POINT** that if you want to express this gene, you need to clone only exons that produce the protein(coding region).

**Remember bacteria does not have introns in its DNA.

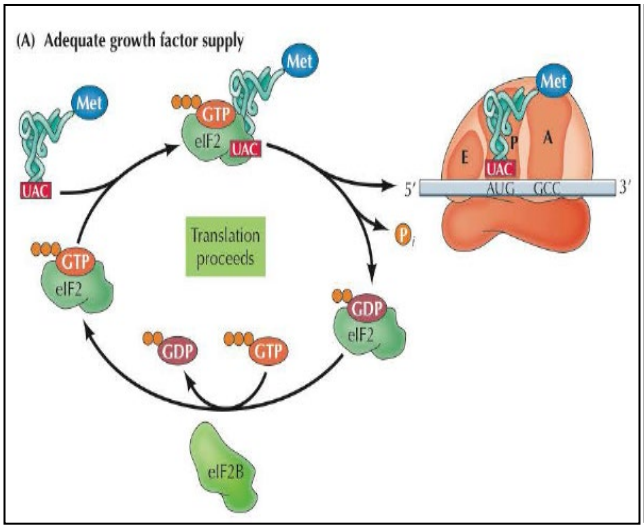


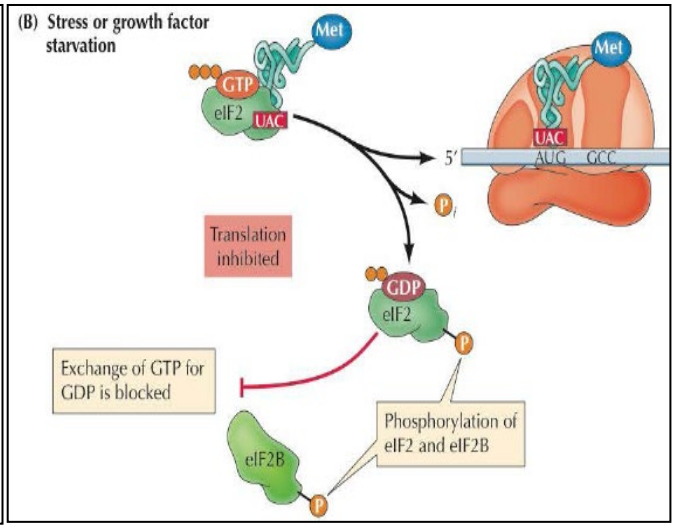


Regulation of translation:

-The translation process id regulated process and can be regulated at different levels: global level and specific level.

Global regulation:





Previously we talked about degeneration and recycling of eIF2 and this is very important in the initiation of translation, the function of eIF2 of bringing tRNA with the methionine amino acid to the small ribosomal subunit to initiate the translation, so here we have adequate and enough supply of essential amino acids and growth factors, so continuous activation and deactivation of eIF2 lead to continuous translation process ***while*** stress and low essential amino acids and growth factors supply will lead into inactivation of the eIF2 and there's no more initiation of translation and then translation is inhibited.

Heme and protein synthesis: (specific example of global regulation)

-Heme: part of hemoglobin (which is kind of protein found in our red blood cells and responsible for transporting of oxygen from the lung to the tissues and CO2 from the

tissues to the lung) and it is a complex of porphyrin (which heterocyclic organic compound) and ferrous iron (Fe+2), responsible of the red color of our blood.

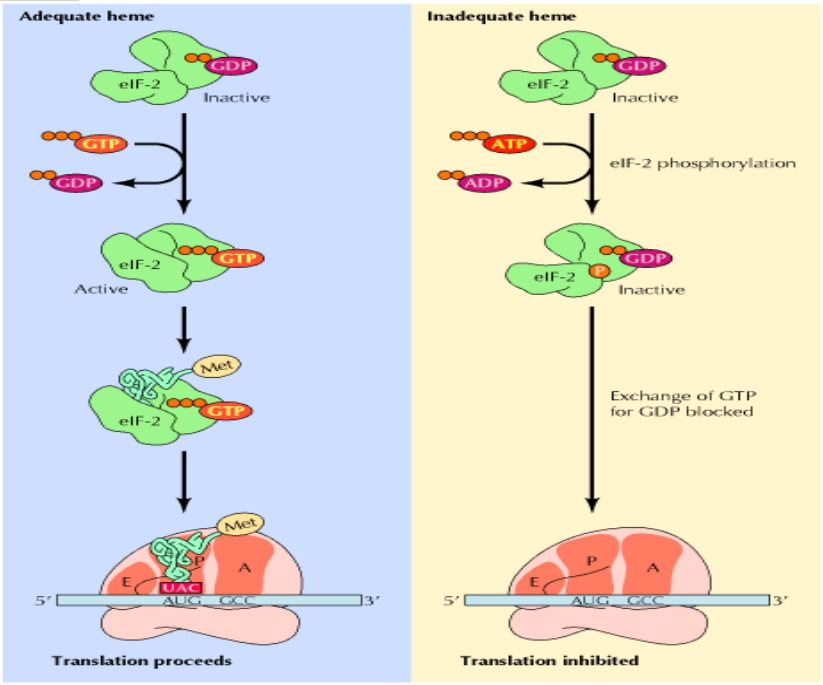
1-First, in reticulocytes(immature erythrocytes which are type of cells in our bone marrow),heme stimulates protein synthesis -synthesis of hemoglobin occurs in these cells.

2-The mRNA is translated only if adequate heme is available to form functional hemoglobin molecules.

3-This is done via regulating the activity of eIF-2, which is responsible for escorting initiator methionyl tRNA to the ribosome.

4-eIF-2 must be bound to GTP to be active. When it is released from the ribosome, GTP is hydrolyzed to GDP, which must be exchanged with GTP for eIF-2 to be active again.

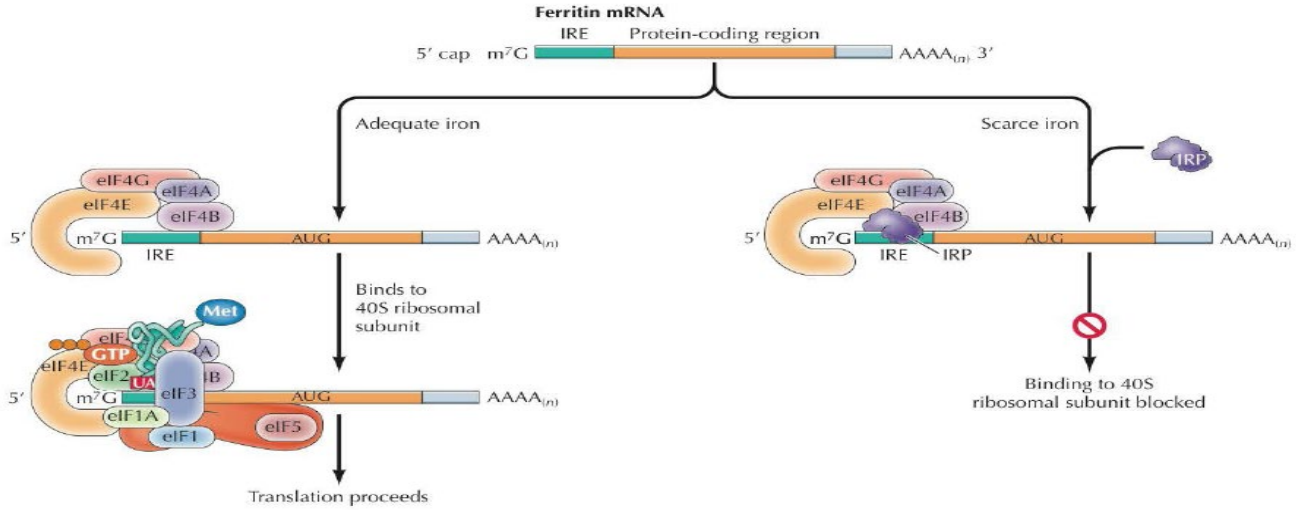
-To understand better let's see this figure:



1- If adequate heme is available eIF2 can be activated, deactivated and recycled, GDP-GTP exchange occurs and translation is able to proceed. (Initiation of translation)

2- If heme supplies are inadequate translation will be inhibited because there's no activation and recycling of eIF2, a protein kinase that phosphorylates eIF-2 is activated. Phosphorylation of eIF-2 blocks the exchange of GTP for GDP, so eIF-2/GTP cannot be regenerated and translation is inhibited. (NO initiation of translation)

Ferritin:

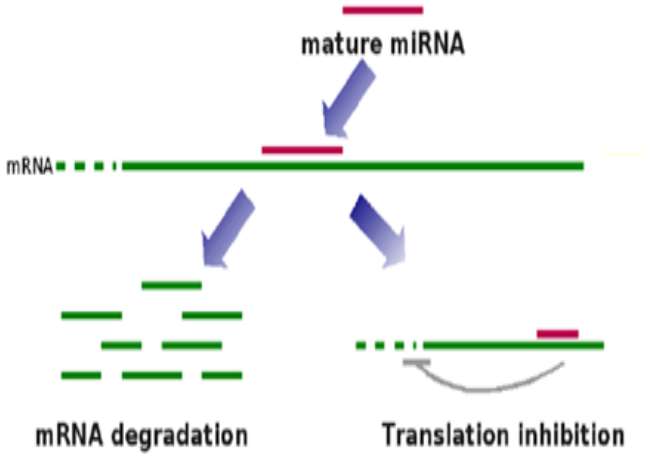


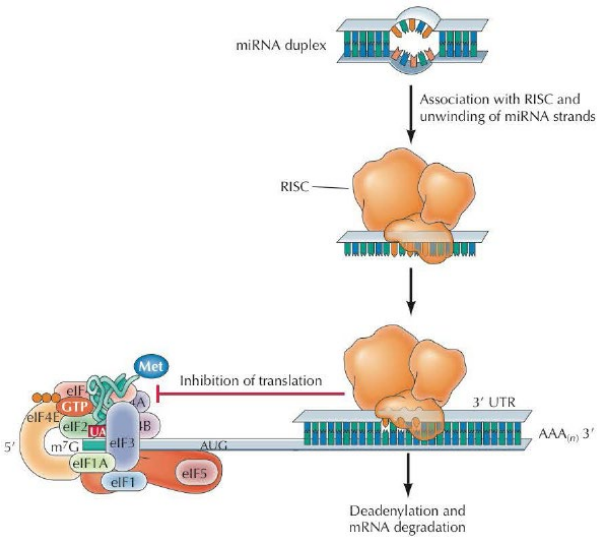
Another example is ferritin expression:

There's a relationship between the **iron conc.** and **ferritin expression**, in case we have low amounts of iron in our body that means that there's no iron to bind to a specific protein called iron responsive binding protein and this protein once it's free without iron, it can go to iron responsive element at the untranslated region at the 5' end of the ferritin mRNA and once it binds to the iron responsive element it can stop the initiation of the translation **while** when we have adequate amount of iron then the iron will bind to the iron responsive binding protein and inhibit its binding to the iron responsive element at the 5' end of the ferritin mRNA so the translation can proceed and produce more ferritin protein.

Regulation by microRNA (miRNA):

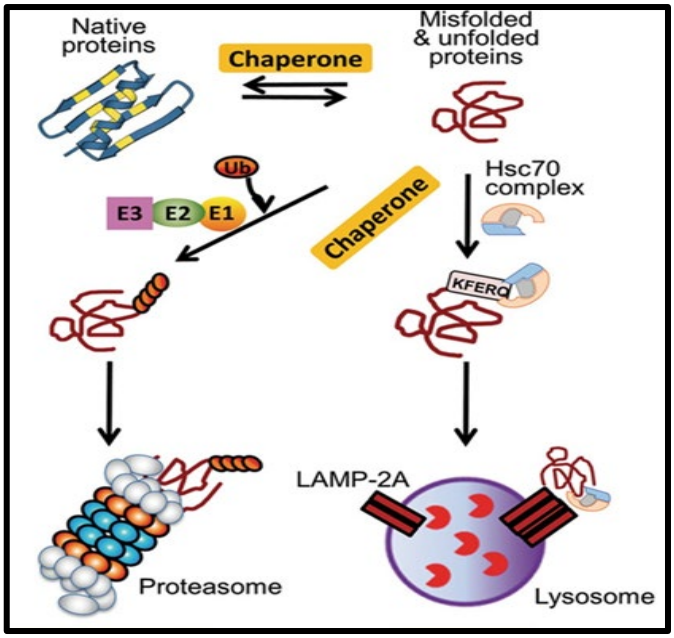
miRNA: a short non-coding RNA synthesized by RNA Pol II into single-stranded, primary miRNA (pre-miRNA) transcript which gets processed and one strand loaded into RISC (RNA induced silencing complex) complex where miRNA is targeted to the 3'-UTR of mRNA and the miRNA can be complementary to mRNA -once it finds this complementary sequence- then the RISC complex can induce the degradation of the mRNA and inhibit the translation process.





Fate of (mis)- and (un)-folded proteins:

You need to remember that there's relationship between the structure and function of any protein which means that a protein should have a specific structure folded to do the proper and right function which should require proper folding, so in our cells there's a kind of proteins that regulate the proper folding of proteins called chaperons but in case we have mis and unfolded proteins that need to be removed by cells → through the degradation by proteases.



, this process occurs in either in degradative subcellular organelles like lysosomes (rich in proteases and have very low PH) or by the macromolecular **proteasomes** (they are complex of proteins that can degrade the unneeded of mis folded proteins by a process called **ubiquitinylation** which involves labeling mis folded proteins by small polypeptides known as ubiquitin).

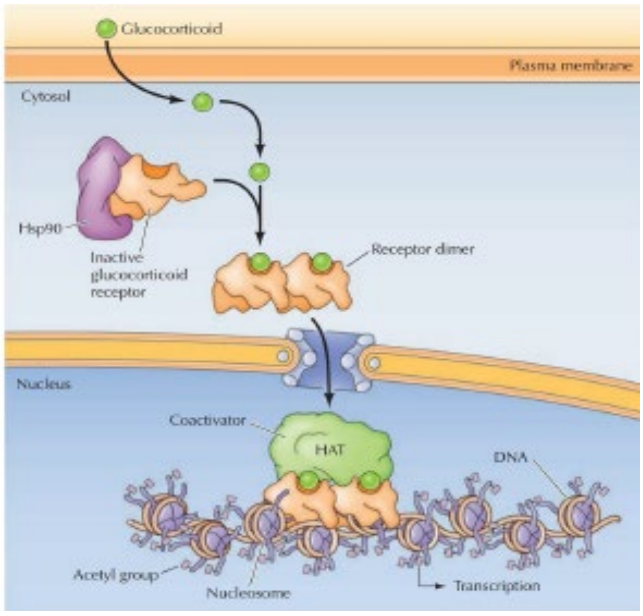
****After the degradation process there will be free amino acids that can be used by cells again for the synthesis of new proteins so they won't be a waste.**

Connecting outside to inside: from cell signaling to protein synthesis

how can the cell transfer the signal from the outside to the inside of the cell? And how such signal can regulate the protein synthesis?

There are different modes of regulating protein synthesis

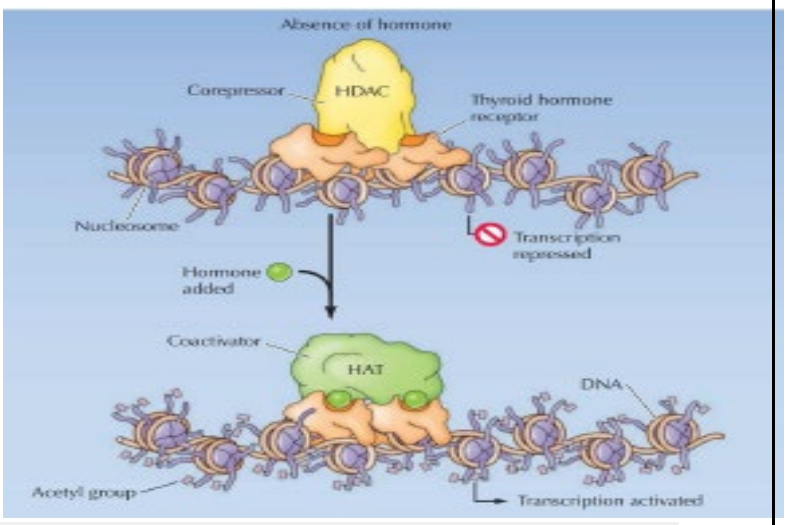
1-Glucocorticoids:



Glucocorticoid are steroid hormones, like androgens and other steroid hormones, diffuse across the plasma membrane and bind to the glucocorticoid receptor.

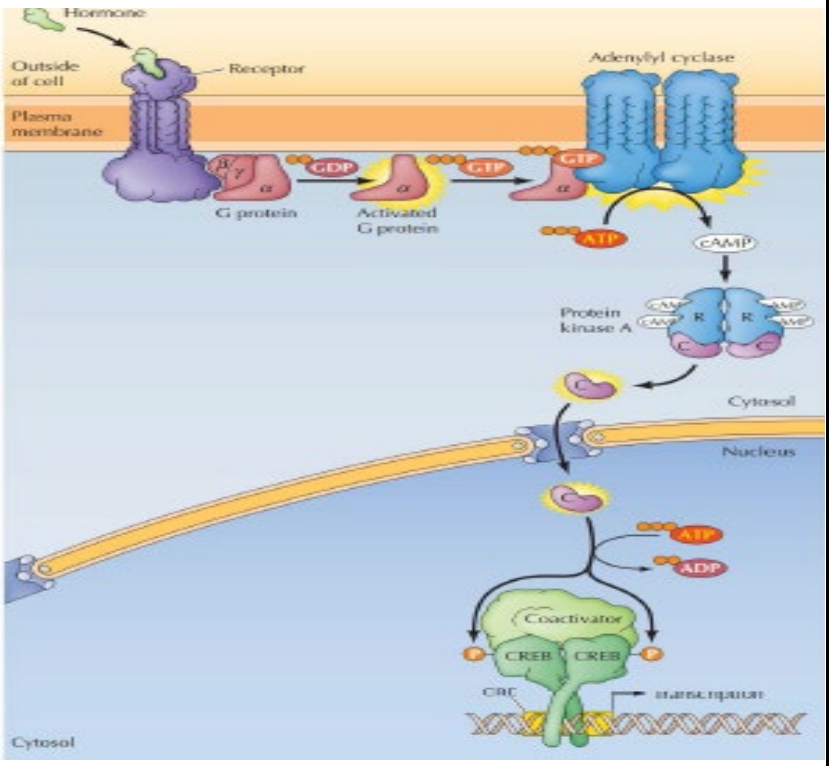
Glucocorticoid binding allows the formation of receptor dimers (causes dimerization of glucocorticoid receptor and this activates glucocorticoid receptor), the activated receptors translocate to the nucleus, bind DNA, and associate with coactivators (example: histone acetyltransferase (HAT)) to stimulate transcription of their target genes.

2- Thyroid hormone:



Thyroid hormone receptor binds DNA in either the presence or absence of hormone, In the absence of hormone, the receptor associates with corepressors with histone deacetylase (HDAC) activity. In the presence of hormone, the receptor associates with coactivators with histone acetyltransferase (HAT) activity, and this will induce and activate the transcription of target genes -so it's a little bit different that glucocorticoid mechanism of action.

cAMP-inducible gene expression:



Another way of signaling that regulate gene expression is c-AMP second messenger ,this pathway occurs through the binding of certain hormones to their receptors on the cell membrane followed by the activation of G-protein what activates Adenylyl cyclase what catalyze the conversion of ATP into cyclic AMP and activation of protein kinase A.

The catalytic subunit of protein kinase A is freed, translocate into the nucleus, and phosphorylates the transcription factor CREB (CRE-binding protein), leading to the recruitment of coactivators and expression of cAMP-inducible genes.

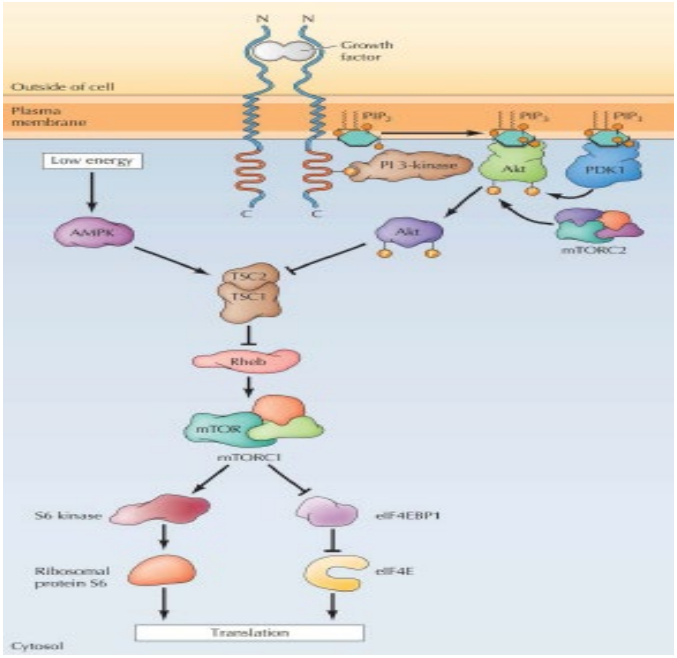
The mTOR pathway: Coupling growth to energy stores

Another example is the mTOR pathway which is very important in cell preformation and regulation of different genes expression, also mTOR is very important for cancer treatment.

)

mTOR represents an interesting example of a coupling of growth to the energy stores (by logic when we don't have enough energy so your cell don't divide to

keep the energy for other important processes and Pathways) .mTORC1 stimulates translation by phosphorylating 56 kinase (which phosphorylates ribosomal protein 56) and by phosphorylating eiF4E binding protein-1 (4E-BP1), relieving inhibition of translation initiation factor eiF4E.



-At high energy, Akt is activated leading to activation of mTORC1 and, hence, translation.

-At low energy stores (AMP>ATP), AMP kinase (AMPK) inhibits translation by inhibiting mTORC1.

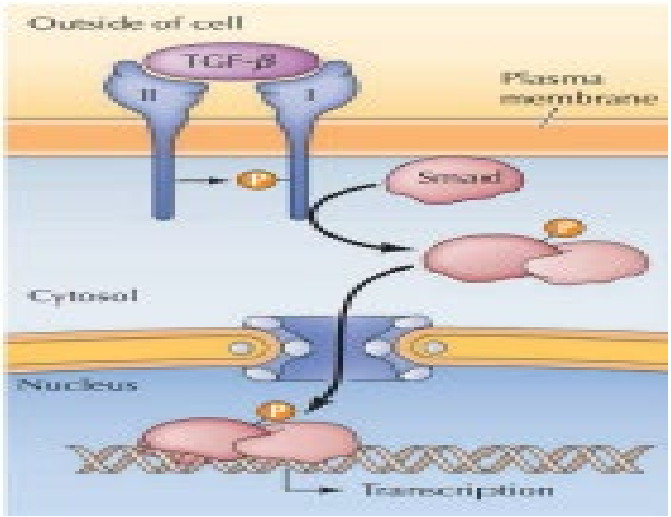
The MB kinase inhibits the translation by inhibiting the mTORc1, so as we see when we have low energy the mTOR is inhibited and there's no translation (when we have enough energy mTOR is activated and the translation occurs) so translation depends on the level of energy inside the cells

Direct activation of transcription factors:(TGF-β receptors→ Smad)

sometimes there's a possibility for the direct activation of transcription factor, so there's no need for intermediate in the gene expression like TGF-β receptors.

1-When bound to their ligand, transforming growth factor receptors (TGF-β receptors) phosphorylate a Smad protein.

2- Phosphorylated Smads form complexes and translocate into the nucleus to activate transcription of target genes.

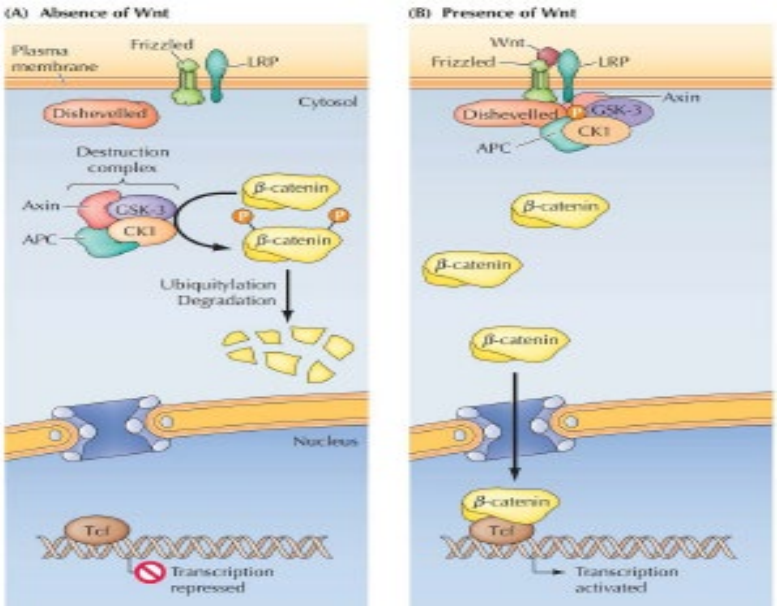


So it occurs in very direct way the transforming growth factors beta receptors once they activate the Smad , it'll go to the nucleus and activate the transcription.

Trapping of transcription factors: (The Wnt pathway)

Another way that regulate gene expression is **trapping transcription factors**.

there's an important protein that is linked to the **Wnt** which is called beta catenin (protein by which **Wnt** mediate its pathway)



(A) In the absence of the ligand **Wnt**, β-catenin is ubiquitylated and degraded so the beta catenin will be deactivated and there will be no transcription.

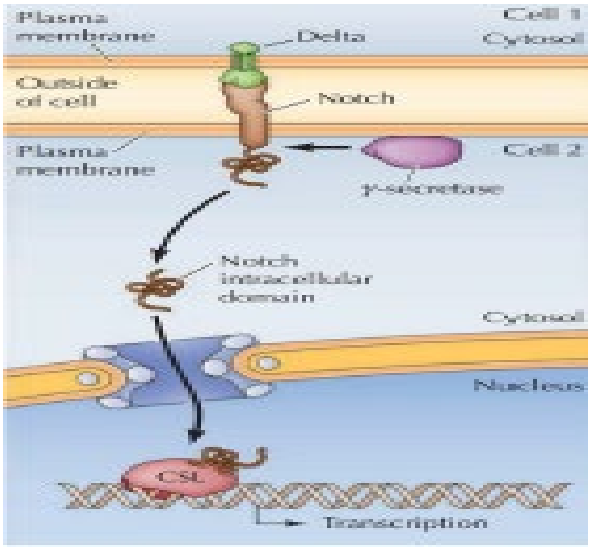
(B) When **Wnt** is present, the destruction complex is inactivated and β–catenin is stabilized, translocates into the nucleus and forms a complex with other transcription factors activating transcription.

So as we see in the presence of mediator protein -that meditate the transferring signal to the new class- stay stable, **but** in the case of the absence of ligand and the mediator protein that will degraded and no more transcription occur.

The TF is within the membrane receptor: (The Notch pathway)

Another mechanism that regulate gene expression through signaling pathways is the binding of the receptor and its Ligand in 2 different cells, for example notch receptors.

The binding of its ligand leads to proteolytic cleavage of Notch by y-secretase. This releases the Notch intracellular domain, which translocates to the nucleus and interacts with a transcription factor to induce gene expression.



So as you can see here that this signaling pathway is mediated between two cells in previous examples we mentioned that the signaling pathway can cross the cell membrane and find its receptor inside the cytoplasm or inside the nucleus or flow binding to receptors on the surface of the cell but here a different example that the interaction between the two cells that mediate the signaling pathway and mediate the regulation of expression of target genes.

Levels of regulation:

- Transcription
- RNA processing
- RNA transport
- mRNA stability
- Translation
- Post-translational modification
- Protein activity
- Protein degradation

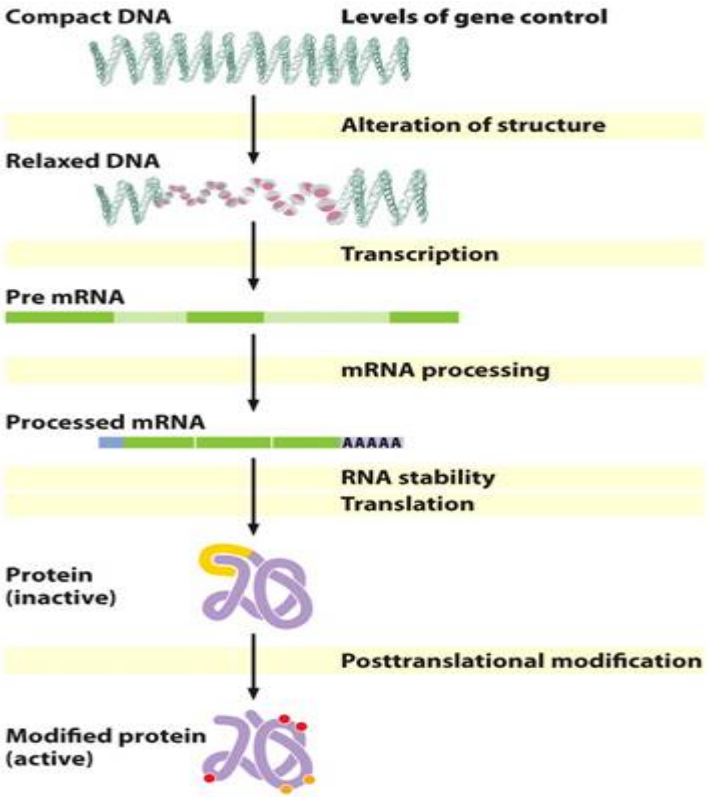


Figure 16.1
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The End 😊