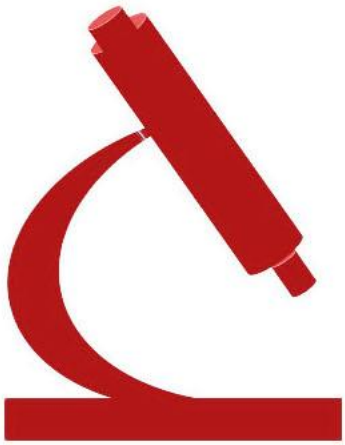

CYTOLOGY

Premed 2018 - JU

◉ Sheet ○ Slides

Number

Molecular 21

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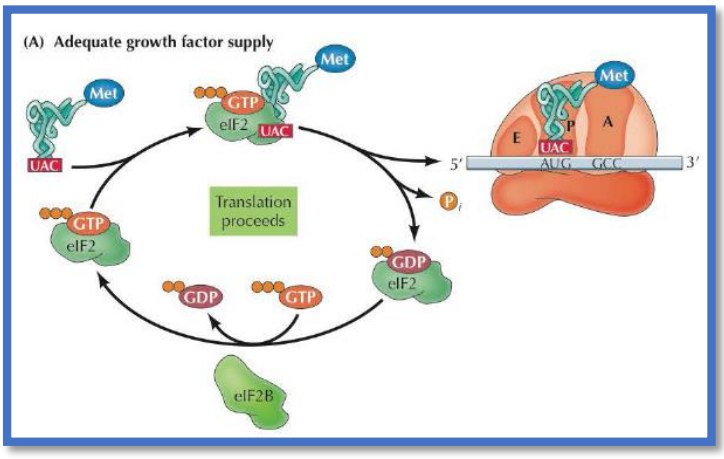
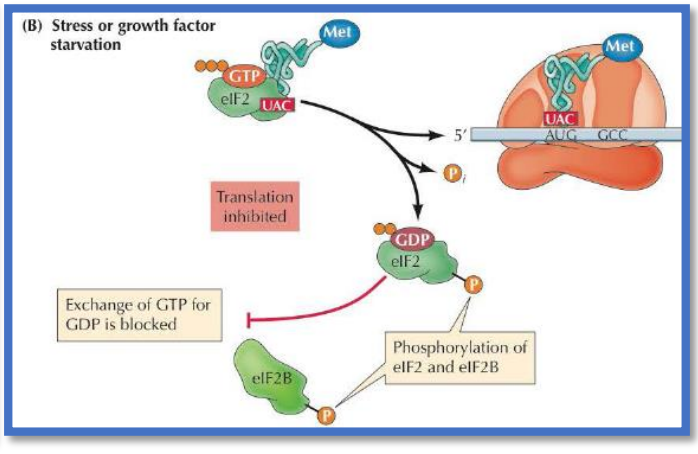
➢ Global Regulation of Translation

- Regulation of all translation processes in the cell is achieved by regulating initiation factors, which regulate all activities of translation.

➢ Regulation of eIF2:

- ✓ eIF2 is an important factor for the initiation of translation.
- ✓ Remember that more than one ribosome can translate a single mRNA simultaneously forming a polyribosome (Polysome). For each ribosome to translate the mRNA, it needs eIF2 to bring the first tRNA that's attached to methionine in eukaryotes and to N-formylmethionine in prokaryotes.
- ✓ In order for eIF2 to function, it has to be bound to GTP.
- ✓ When it binds to GTP, eIF2 can bring the tRNA carrying the first amino acid and attach it to the AUG codon of the mRNA being translated.
- ✓ After attaching the first tRNA to the mRNA, eIF2 is released from the ribosome and GTP is hydrolyzed to GDP.
- ✓ eIF2 then releases GDP and binds to GTP to activate another round of translation.
- ✓ This process is regulated by **phosphorylation**.
  - When eIF2 gets phosphorylated by a kinase, it can't release GDP and bind to GTP which means that it can't initiate another round of translation (translation is inhibited).
  - In order for eIF2 to function again, it needs to be dephosphorylated so that it can release GDP and bind to GTP instead.



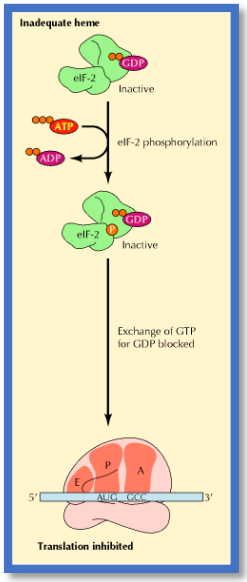


➢ Heme & Regulation of Protein Synthesis:

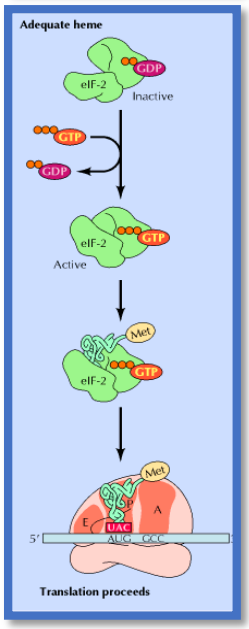
- ✓ As said before, we can regulate all activities of translation in cells. This is particularly true for reticulocytes.
- ✓ Reticulocytes are immature erythrocytes (immature red blood cells).

- ✓ The primary function of red blood cells is to carry oxygen by hemoglobin so these reticulocytes need to synthesize a lot of hemoglobin.
- ✓ In order to synthesize hemoglobin, reticulocytes need two things: the heme and the globin protein.

Now the question is: If there's no heme, why should reticulocytes bother and synthesize globin since they're not going to use it to form hemoglobin?

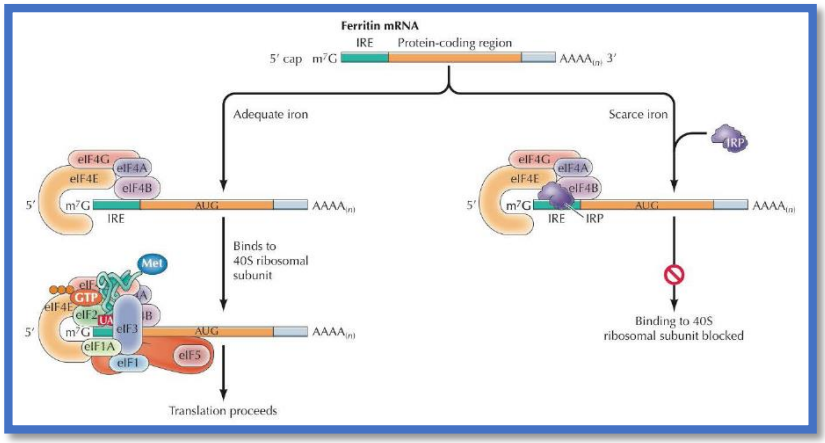


- ✓ If there's a deficiency in heme, synthesis of globin must stop.
- ✓ If heme supplies are inadequate, a protein kinase is activated to phosphorylate eIF2 which means that it won't be able to release GDP and bind to GTP → eIF2 can no longer initiate the translation of the mRNA of the globin protein.
- ✓ All reticulocytes do is synthesize globin so if heme isn't available, all activities of translation in these cells stop.
- ✓ If adequate heme is available, eIF2 won't be phosphorylated and it can release GDP and bind to GTP and now it can bring the first tRNA to the ribosome initiating the translation of the globin mRNA to form functional hemoglobin molecules.



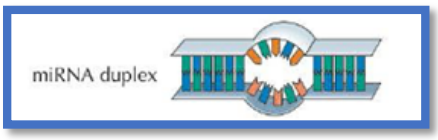
➢ Ferritin

- ✓ *Remember!* When there's low iron concentration, IRE-binding protein binds to the iron response element (IRE) in ferritin mRNA to inhibit its translation.
- ✓ IRE-binding proteins inhibit ferritin mRNA translation by binding to the 5' UTR preventing ribosomes from binding to the 5' UTR.
- ✓ IRE-binding proteins must be released in order for translation to take place.

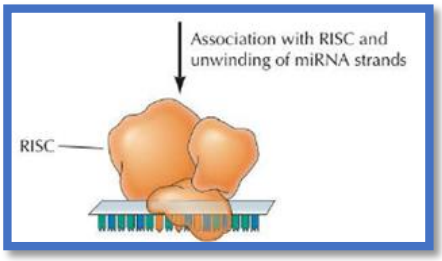


➢ Regulation by microRNA (miRNA)

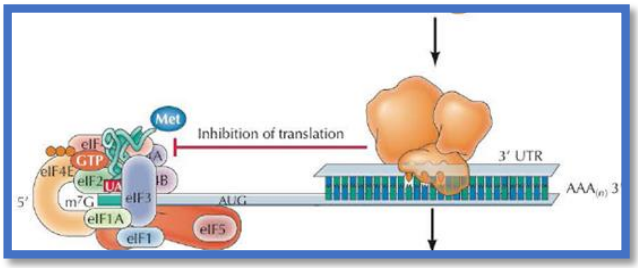
- ✓ MicroRNAs are synthesized by RNA poll II into single stranded, primary miRNA (pri-miRNA) transcript but by a certain mechanism, they form a miRNA duplex (become double stranded).



- ✓ MiRNAs are associated with a protein called RISC. RISC binds to one of the miRNA's strands and attaches it to a complementary 3' UTR of an mRNA strand.



- ✓ When miRNA hybridizes with the 3' UTR of an mRNA molecule, either the mRNA gets degraded or translation is inhibited.



- ✓

- ✓ Consequently, miRNA decreases the level of protein production in the cell.

- This shows the importance of UTRs. Even though they aren't translated, they're still important because they could contain a regulatory sequence.

➢ Regulation at Protein Level

- ✓ Unfolded and misfolded proteins must be degraded.
- ✓ Cells degrade unfolded and misfolded proteins by different mechanisms.
- ✓ Proteins are degraded either in degradative subcellular organelles like lysosomes or by the macromolecular **proteasomes**.
- ✓ Proteins get degraded by proteasomes and the amino acids are recycled and can be utilized again in the synthesis of another protein.

Now the question is: How are proteins targeted to be degraded by proteasomes?

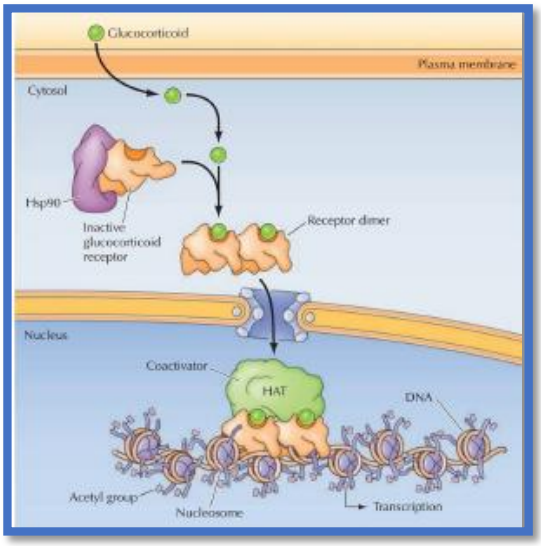
- ✓ Proteins are targeted for destruction in a proteasome by **ubiquitinylation.**
- ✓ Ubiquitinylation is the process of labeling proteins by ubiquitin.
- ✓ Ubiquitins are small lipophilic polypeptides that label proteins that are targeted for degradation by proteasomes.

➢ Connecting Outside to Inside: *from cell signaling to protein synthesis*

- This part should give you an insight on how what happens outside the cell (for example: change in concentration of certain molecules) could affect the transcription that happens inside the cell.

➢ Glucocorticoids

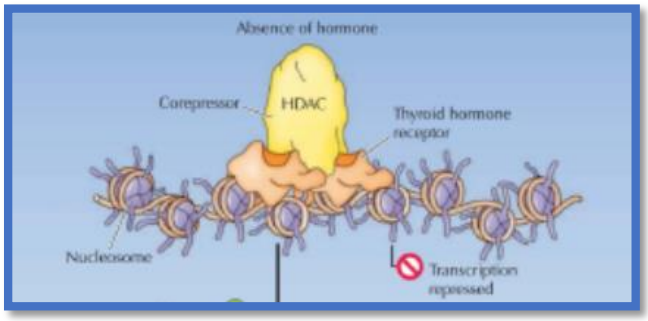
- ✓ Glucocorticoids are small lipophilic hormones that diffuse easily across the plasma membrane.
- ✓ These hormones bind to glucocorticoid receptors causing them to dimerize.
- ✓ Once dimerized, these receptors are translocated into the nucleus and bind to a consensus sequence known as Hormone Response Element (HRE).
- ✓ Binding of these receptors to HRE leads to the recruitment of coactivators.
- ✓ These coactivators (for example: **h**istone **a**cetyl**t**ransferase **HAT**) have enzymatic activity and can modify the DNA or histones, stimulating transcription of their target genes.



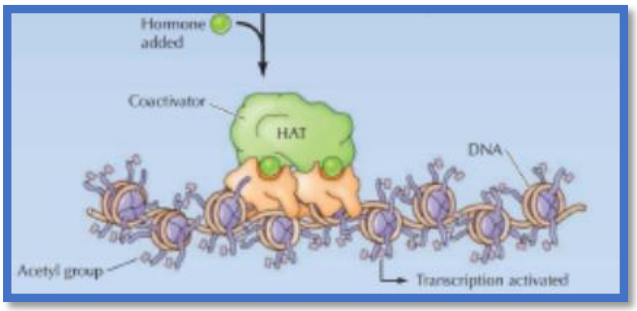
- We can see now that the increase in glucocorticoid's concentration outside the cell leads to the stimulation of transcription of a certain gene inside the cell.

➢ Thyroid Hormone

- ✓ Thyroid is a small lipophilic hormone that diffuses easily across the plasma membrane.
- ✓ Thyroid hormone receptor is always bound to DNA in either the presence or absence of hormone.
- ✓ In the absence of thyroid, thyroid receptor is associated with histone deacetylases **HDAC** (corepressors) which remove acetyl groups from histones, strengthening the interaction between DNA and histones leading to the condensing of DNA and inhibition of transcription.

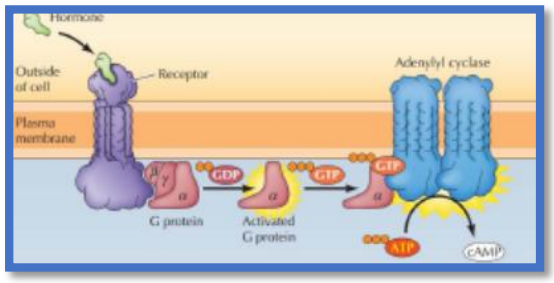


- ✓ When thyroid binds to the receptor, the receptor releases histone deacetylases **HDAC** (corepressors) and binds to histone acetyltransferases **HAT** (coactivators). HAT acetylates histones, weakening the interaction between DNA and histones leading to the de-condensing of DNA and activation of transcription.

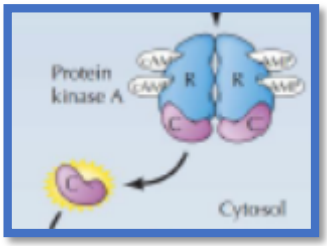


➢ cAMP-inducible Gene Expression

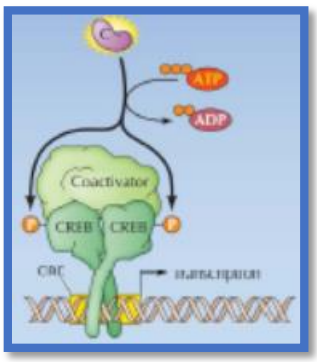
- ✓ When a certain hormone (for example: norepinephrine) binds to its receptor (G-protein coupled receptor), a G-protein is activated. The G-protein then activates an enzyme called Adenylate Cyclase which catalyzes the production of cAMP.



- ✓ cAMP is a second messenger. It binds to protein kinase A **(PKA)** causing it to dissociate into 4 subunits (2 regulatory subunits and 2 catalytic subunits).



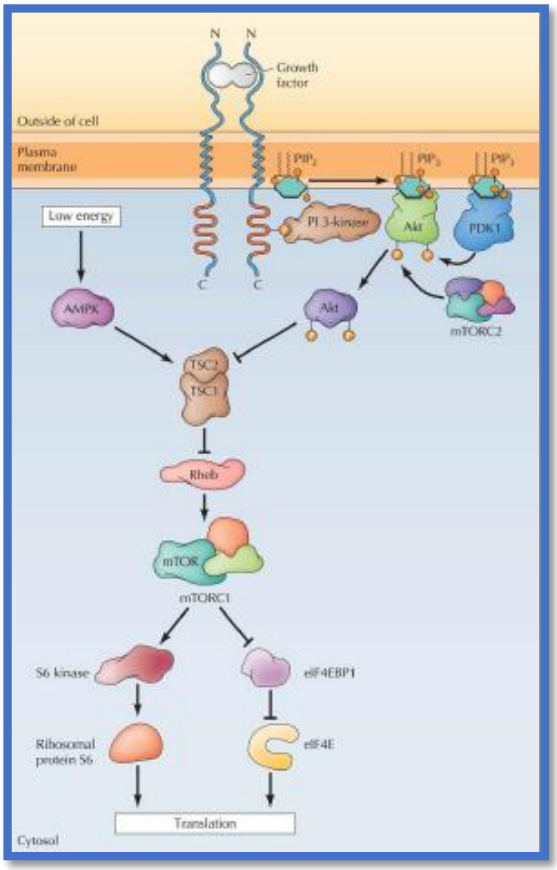
- ✓ The catalytic subunits of PKA go into the nucleus and phosphorylate transcription factors including a transcription factor known as **CREB** (cAMP Response Element Binding Protein) allowing it to bind to a consensus sequence known as **CRE** (cAMP Response Element) leading to the recruitment of coactivators and expression of cAMP-inducible genes.



➢ mTOR Pathway: *coupling growth to energy stores*

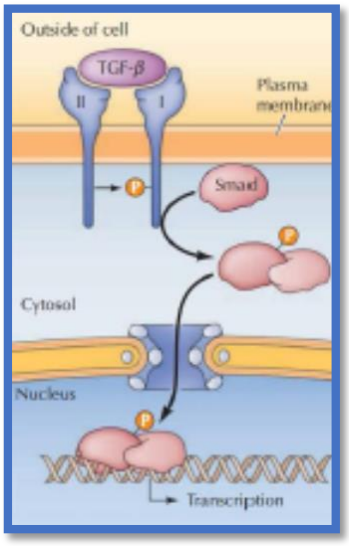
- ✓ mTOR pathway can be regulated by two different molecules depending on the availability of nutrients.
- ✓ If there's insufficient amount of nutrients (low energy stores) which means there's more AMP than ATP, AMP activates a kinase known as AMP kinase.
- ✓ The activation of AMP kinase inhibits translation by inhibiting mTORC1.

- ✓ If the cell has sufficient amount of nutrients (high energy stores), Akt is activated leading to the activation of mTORC1 and, hence, translation.



- ✓ **[Wasn't mentioned in the lecture; Only mentioned in the slides]**
  →mTORC1 stimulates translation by:
  1. Phosphorylating 56 kinase which phosphorylates ribosomal protein 56.
  2. Phosphorylating eiF4E binding protein-1 (4E-BP1), relieving inhibition of translation initiation factor eiF4E.

➢ **TGF-β Receptors:** *direct activation of transcription factors*

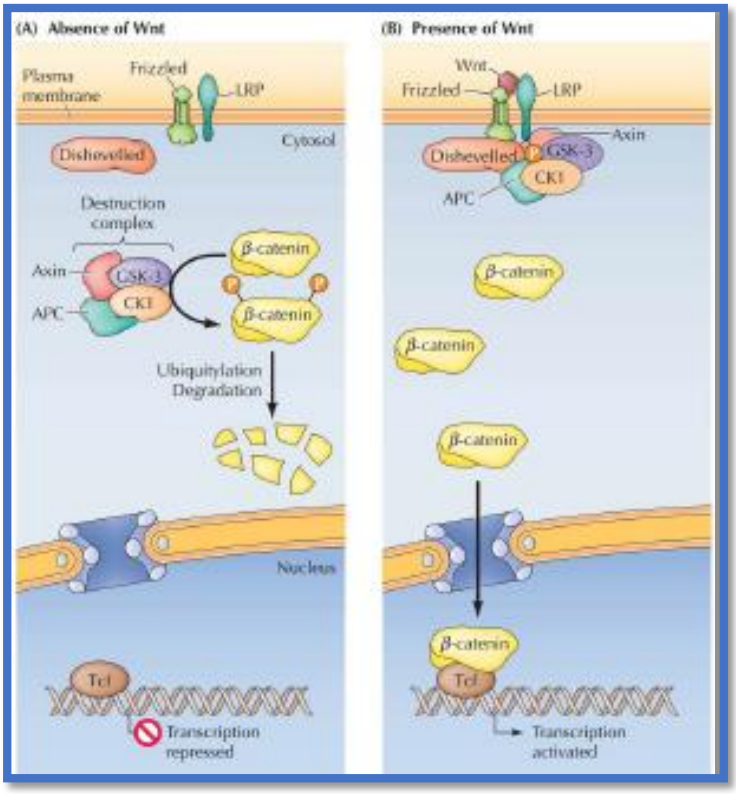


- ✓ Smad is a transcription factor that's activated **directly** by TGF-β receptors.
- ✓ When a specific ligand binds to TGF-β receptors activating them, these receptors phosphorylate a Smad protein (transcription factor).
- ✓ Phosphorylated Smads form complexes and translocate into the nucleus to bind to the DNA and activate transcription of target genes.

➢ **The Wnt Pathway**

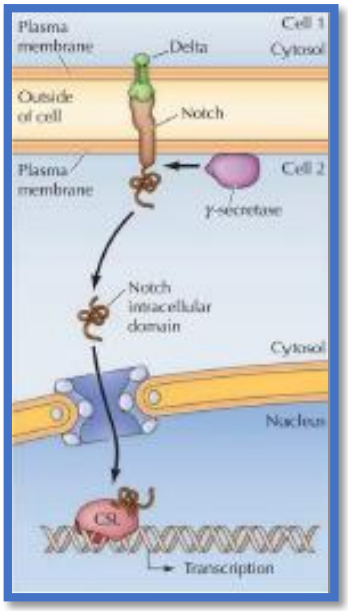
- ✓ This pathway is important for development and differentiation.
- ✓ In the absence of the ligand Wnt, β-catenin is ubiquitylated and degraded.
- ✓ When Wnt is present and the Wnt pathway is activated, the destruction complex is inactivated and β-catenin is stabilized.
- ✓ When stable, β-catenin translocates into the nucleus and forms a complex with other transcription factors activating transcription.
- ✓ In the Wnt pathway, β-catenin acts as a coactivator of transcription.

- *This figure illustrates how the presence of Wnt affects the stabilization of β-catenin.*



➢ The Notch Pathway

- ✓ In this pathway, the transcription factor, Notch, is part of the receptor.
- ✓ When the pathway is activated, an enzyme called y-secretase cleaves (proteolytic cleavage) Notch, releasing the Notch intracellular domain from the receptor.
- ✓ Once Notch is released from the receptor, it translocates to the nucleus and interacts with a transcription factor to induce gene expression.



➔ Notes:

- **During the lecture, Dr Ma'moun said that Glucocorticoids and cAMP pathways could either activate or inhibit transcription, but we only mentioned what's written in the slides.**
- **We aren't required to know the mediators of each pathway.**